THE PHYSICOCHEMICAL AND PHARMACOKINETIC STUDIES OF SOME PAIN RELIEF DRUGS

by

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Some questions are raised and errors noted
The internal examiner / candidate may like to
review these if corrections are to be made

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ABSTRACT

Physicochemical and pharmacokinetic studies of some pain relief drugs

Sensitive, selective and accurate analytical techniques based on gas-liquid chromatography (with 3% W/W SP 2250 column linked to a nitrogen sensitive detector) have been developed which enable the determinations of bupivacaine, a family of six local anaesthetics and pethidine with its major metabolites in human biological fluids. Using these useful procedures, the physicochemical properties, clinical pharmacokinetics and metabolism of the drugs have been fully determined.

Kinetic analyses in the investigation of the effect of adrenaline (1:200,000) on bupivacaine indicated the addition of vasoconstrictor had no subsequent effect on the pharmacokinetics of bupivacaine. However, the mean peak plasma concentration was significantly lower (2.57±0.61 and 3.22±0.27 μg/ml with and without adrenaline respectively) and the mean time for maximum absorption was longer (24.2±2.7 and 15.0±1.3 min respectively) in the adrenaline group. The results showed that the addition of adrenaline appeared prudent to minimize bupivacaine toxicity.

An examination of physicochemical properties and buccal absorptions of six local anaesthetics (amethocaine, bupivacaine, etidocaine, lignocaine, mepivacaine and prilocaine) has revealed the onset of action and the duration were shown dependent on the pKa values and the logarithms of partition coefficients (K) in n-hexane-Sϕrensen buffer system. The buccal absorption test supplemented by the n-hexane-buffer partition coefficient could be used as a reference for the ability of a local anaesthetic molecule to penetrate biological barriers.

Studies on the plasma concentrations of pethidine and norpethidine in patients have shown that pethidine was absorbed rapidly and reached its peak concentration within 2 hours after intramuscular administration. The metabolite, norpethidine, appeared in the plasma from 0.25 to 0.75 hour. The comparison of pharmacokinetic profiles of pethidine and norpethidine among Caucasian, Chinese and Nepalese showed that Asian patients did not eliminate pethidine as effectively as the Caucasians but they produced more norpethidine which stayed longer in the plasma.

The percentage of protein binding ranged from 72±2.8 to 43±2.7% and from 57±3.0 to 27±3.9%, respectively, at various concentrations of pethidine and norpethidine in the protein binding study. Albumin was found to be the

major plasma component responsible for drug binding. The binding affinities of pethidine were comparatively higher than those of norpethidine in all circumstances. There was no direct displacement effect on the binding between pethidine and norpethidine, indicating these two compounds exhibited no displacement action on the binding site to one another. The mean pethidine bound in Caucasian, Chinese and Nepalese patients at about 100 ng/ml pethidine was similar, implying the absence of interethnic protein binding variation.

The determination of pethidine and its major metabolites in urine samples showed that these compounds excreted in the urine were varied remarkably among subjects. The mean 24 hours urinary recoveries in eight Chinese patients of pethidine, norpethidine, pethidinic acid, norpethidinic acid, glucuronides of pethidinic and norpethidinic acids were 6.62±5.05, 4.33±1.19, 18.9±6.29, 9.10±4.26, 15.1±3.02 and 7.57±2.28% respectively. It showed that the major metabolic pathways of pethidine in the eight patients were hydrolysis followed by conjugation. Over 60% of the dose was accounted for in 24 hours after intramuscular administration of 1 mg/kg of pethidine.

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CHAPTER 1

GENERAL INTRODUCTION

1.1. PLANS FOR THE PRESENT INVESTIGATION

The present work concerns the studies of physicochemical properties and pharmacokinetics of pain relief drugs. The drugs that were examined in the studies fall into two major categories: local anaesthetics and narcotic analgesics. The main reason of choosing these compounds in our research was because these agents are the most commonly and widely used pain relief drugs in Hong Kong as well as many other overseas countries, particular in obstetrics and controlling post-operative pain. In general, the study involved the collaboration with physicians in hospitals (The Prince of Wales Hospital and The British Military Hospital of Hong Kong) for obtaining plasma or urine samples from patients who had usually undergone various kinds of surgery. The whole course of the research initially required the development of novel chromatographic methods as well as the improvement of present available chromatographic methods and thereafter, these methodologies were applied to ~~analyze~~ determine the amount of the parent drug together with its metabolite(s) in the biological samples. Results obtained from these investigations could provide important information on the adequate handling the pharmacokinetic profiles and metabolic patterns of the above named drugs when they were used post-operatively.

The following investigations were intended to carry out in our work: Grammar

1.1.1. Evaluation of the effect of vasoconstrictor on the phamacokinetics of bupivacaine after interpleural administration

The interpleural administration of local anaesthetics is a recently introduced technique to provide post-operative analgesia. Like other new techniques, a number of questions associated were not fully understood and more scientific studies were required to find out some of the answers. One of such questions was the usefulness of co-administrating with vasoconstrictor in the interpleural administration of local anaesthetics. Therefore we propose to develop a simple and accurate GC method which will conveniently measure the plasma levels of bupivacaine and to apply this method to define the actual effect on patients caused by the vasoconstrictor, adrenaline, on the disposition of local anaesthetics. This part of work will be reported in Chapter 2.

1.1.2. Simultaneous gas-liquid chromatographic determination of some clinically used local anaesthetics drugs and their physicochemical properties

The analytical assay will be based on the modification of the method in determining bupivacaine. We plan to use this assay to measure the lipid solubilities and buccal absorptions of the compounds simultaneously and to examine whether there is any correlation between these characteristic properties and the pharmacological activities, such as drug potency, duration and onset of action, of these local anaesthetics. This part of work will be reported in Chapter 3.

1.1.3. Gas-liquid chromatographic determination of plasma pethidine and its metabolite, norpethidine in Chinese patients after intramuscular administration

Norpethidine, the only metabolite of pethidine that can be found in the blood circulation after administration of the parent drug, ~~could~~ can produce seizures~~, effect on patients~~. The chromatographic property of this compound is poor, the previously reported GC methods were tedious, and always required sample derivatization. Further, the pharmacokinetic data ~~of~~ on pethidine and norpethidine in the Chinese population was relatively scarce. Therefore we would like to develop a simple and useful GC method to determine these compounds in the plasma of Chinese patients and the data so obtained will be compared with those from other ethnic groups. This part of work will be reported in Chapter 4.

1.1.4. Gas-liquid chromatographic determination of pethidine and all its major metabolites in urine samples

Apart from norpethidine, other metabolites such as pethidinic and norpethidinic acids, conjugates of pethidinic and norpethidinic acids (formed by the conjugation reaction of a glucuronide group to the corresponding acid) respectively, can also be found in urine. The chromatography of these metabolites has not been ~~successfully~~ reported. Therefore we would like to develop a GC method to measure these drugs in the urine of patients after pethidine administration, which should help ~~us~~ to elucidate the disposition of pethidine more clearly among individuals and different ethnic groups. This

part of work will be reported in Chapter 5.

1.1.5. Study of the plasma binding characteristics of pethidine and norpethidine

There is little information on the binding of these two compounds in the literature, especially that for norpethidine was not plentiful. The aspects of protein binding behaviour of pethidine and norpethidine, using incubation and dialysis experiments, will therefore be included in our study. We shall examine the binding properties of these two agents to various isolated protein components as well as to the plasma proteins from patients and healthy subjects. The binding activities of a number of clinical drugs have shown to be altered or influenced by its metabolite. Hence we shall also look at the effect of this type by performing displacement binding experiments between pethidine and norpethidine and the results will probably show us the nature of the binding sites involved. This part of work will be reported in Chapter 6.

1.1.6. Investigation of the existence of different pethidine metabolic pathways among different ethnic groups

A recent report showed that the metabolic routes of pethidine were different between Chinese, Indian and Caucasian healthy subjects and hypothesized the Asian groups were better N-demethylators whereas the Caucasian was better hydrolyzers. We thought we could extend our work using the analytical method that has been developed in Section 1.5.3., to study the plasma pethidine and norpethidine in the three groups of patients (viz,

Chinese, European and Nepalese) and to verify if the recent observation was also apparent in patients. We will also further examine whether the presence of plasma protein binding variability of pethidine and norpethidine exist among these three ethnic groups. This part of work will be reported in Chapter 7.

1.2. CLASSIFICATION OF PAIN RELIEF DRUGS

Pain is a subjective experience and can either be produced by an external or endogenous stimulus. If pain is continuous and severe, disorders such as fear and anxiety may be aroused. In man, these are important components of the perception of pain and treatment for anxiety may be as important as relief of pain. Pain can be alleviated by specific therapy and the drugs used in such process are called analgesics. The choice of analgesics depends upon both the source and the severity of the pain. Strong analgesics that act on pain perception within the central nervous system are grouped as narcotic analgesics. They are mainly used to relieve pain originating in the viscera, or arising from severe injuries, burns or neoplasms. The prototype of this class of drugs is morphine. Another class of drug which also produces an intense analgesic effect is the local anaesthetics. These drugs interrupt impulse transmission in all fibres when applied locally near a nerve trunk. In the smallest effective doses, they act relatively selectively on pain fibres and may therefore be termed as local analgesics. Weaker analgesics, which act chiefly by a peripheral mechanism, have little effect on visceral pain or pain due to wounding, but they are useful for the relief of low grade, aching pain. They are called antipyretic analgesics because their actions are usually associated with temperature lowering effect. Most of these type of analgesics are derivatives of salicyclic acid, aniline pyrazolone, isoindazole and anthranilic acid. As far as the present research concerned, this chapter will only deal with the stronger analgesics.

1.2.1. Narcotic analgesics

Narcotic analgesics can be classified into four main groups: the natural opium alkaloids (Gp. I), semi-synthetics (Gp. II), synthetics (Gp. III) and miscellaneous (Gp. IV). Table 1 summarizes these groups with examples of currently used drugs in clinical practice.

Opium is the dried exudate from unripe seed capsules of the opium poppy, *Papaver somniferum*. The natural opium alkaloids, which relief pain as well as promote sleep and induce a general feeling of peace and well-being, have been used for centuries. Pure morphine was isolated in 1816 and its structure was suggested in 1924 which was proved to be correct after the success of total synthesis in 1952. Minor chemical alterations in various functional groups of the morphine molecule produced new semi-synthetic narcotics such as dihydrohydroxycodeinone. Also, attempts to synthesize part of the morphine molecule which possessed analgesic activity resulted in the Gp. IIIa analgesics, the morphinan series and IIIb analgesics, the benzomorphan series. The discovery of morphine antagonists such as nalorphine stimulated investigations on the morphine antagonists from the morphinan and benzomorphan series. Notably, pentazocine was marketed as a non-addictive, potent analgesic. However it was found to produce similar side effects to other narcotic analgesics.

The 4-phenylpiperidine synthetic analogues, Gp. IIIc analgesics, are the oldest synthetic groups. They were developed in the search for an ideal analgesic without any of the undesirable adverse effects. This led to the production of newer and more potent drugs. Pethidine was the first to be

synthesized and newer analgesics of this group currently use include alfentanil, fentanyl, phenoperidine and sufentanil.

The miscellaneous narcotic analgesics are those which include morphine antagonist, antihistamine and major tranquillizers in the same formulation in the hope that inclusion of these drugs may help to reduce the side effects associated with these agents.

Table 1. Classification of narcotic analgesics.

Group	Examples
I. Natural alkaloids	Morphine, Codeine and Papaveretum
II. Semi-synthetics	Diamorphine, Dihydrohydroxycodeinone and Heroin
III. Synthetics	
(a) Morphinans	Levorphanol, Cyclorphan
(b) Benzomorphans	Phenazocine, Pentazocine
(c) 4-Phenylpiperidines	Alfentanil, Fentanyl, phenoperidine
(d) Diphenylpropylamines	Methadone
IV. Miscellaneous	
(a) With opiate antagonists	Levallorphan
(b) With antihistamines	Cyclizine
(c) With tranquillizers	Droperidol

1.2.1.1. Mechanism of action

The mechanisms of action by which morphine and its similar analogues produce analgesia are complex. Part of the analgesic action of morphine probably derives from its power to exert a calming, soothing and fear-relieving effect. Although this effect is generally assumed to occur in the cerebral cortex, the neurophysiologic mechanisms are entirely unknown. However, morphine also elevates the pain threshold measurably and this effect was shown to be mediated by specific morphine receptors located in the central nervous system important for the transport of pain impulses. Since it is unlikely that all mammalian species should have developed receptors for an alkaloid occurring elsewhere but in the oriental poppy, it appeared more probable that the specificity of these receptors is directed at some morphine-like substance in the brain. Several such substances (endorphins) were discovered and all are peptides with a common N terminal group. The endorphins are morphine-like analgesics and it is not yet certain whether the clinically used opioid drugs act by endorphin release or by direct action on morphine receptors.

1.2.1.2. Adverse effects

The cardinal acute toxic effect of narcotics is respiratory depression. Morphine depresses respiration even in normal therapeutic doses and death from this acute poisoning is the result of cessation and breathing. Long-term chronic dosage leads to tolerance and physical dependence which develop rapidly and are often accompanied by psychological or emotional dependence

of compelling intensity. Tolerance, characterized by decreased intensity and shortened duration of the analgesic, euphoric, sedative and respiratory depression effects of morphine, is preceded by a marked elevation in the lethal dose. Individuals who have become tolerant to morphine are also tolerant to other morphine analogues, that is, there is a cross-tolerance between drugs of the same group.

1.2.2. Classification of local anaesthetics

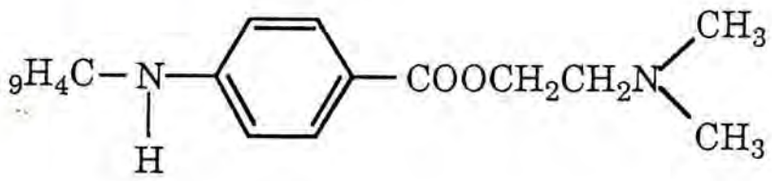
Most useful local anaesthetics are composed of three structural entities: a carbocyclic or heterocyclic aromatic ring (lipophilic portion), an intermediate chain and an amino group (hydrophilic group) as shown in Fig. 1. Such entities can be readily seen in the structural formula for amethocaine. The aromatic ring is essential for anaesthetic activity, and useful injectable local anaesthetics require a delicate balance between the degree of lipid solubility, represented by a particular ring structure, and the potential water solubility of a compound which is this case depends on the presence of the amine group. In addition to the characterization of local anaesthetics on the traditional basis of solubility, most local anaesthetics can be classified chemically as to the type of linkage between the aromatic group and the intermediate chain. One group is represented by the ester linkage susceptible to plasma hydrolysis; another group is the aromatic ring connects to the rest of the molecule by an amide linkage, more resistant to enzymatic inactivation in the blood.

1.2.2.1. Mechanism of action

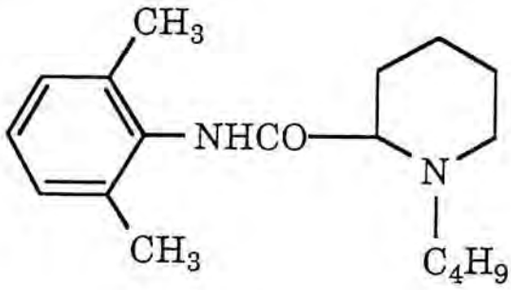
Local anaesthetics generally prevent the generation and conduction of nerve impulses. Their site of action is the cell membrane and the block they produce is the result of interference with changes in membrane permeability to potassium and sodium ions. These permeability changes are responsible for the rising and falling phases of action potential and follow depolarization of the membrane. In the presence of a local anaesthetic the electrical excitability of the tissue gradually decreases until, eventually, complete block ensures.

How local anaesthetics affect the transient changes in ionic permeability is unknown but the potency of these compounds is matched by their ability to increase the surface pressure of monomolecular lipid films. It has been suggested that the anaesthetic squeezes the lipid molecules closer together. In the lipid membrane layers of nerves this could have the effect of closing membrane pores so as reducing ionic permeability; this would have the effect of stabilizing the membrane and reducing its excitability.

Fig. 1 . Comparison of chemical structures of amide (bupivacaine) and ester (amethocaine) types.



Amethocaine



Bupivacaine

Aromatic group Intermediate Chain Amine

1.2.2.2. Adverse effects

Excessive and rapid absorption of these drugs into the general circulation result in nervousness, tremors and convulsions. These effects are very dangerous and are followed by respiratory depression. Nausea, vomiting and abdominal pain may occur sometimes with sudden cardiovascular collapse and respiratory failure for which there is no specific treatment other than respiratory and cardiac resuscitation. Allergic reactions such as rashes, asthma and anaphylactic shock may exist and the subject may be allergic to more than one drug. Local inflammatory or necrotic effects may occurs as these drugs can damage all cells.

1.3. SOME PRINCIPLES OF PHARMACOKINETICS

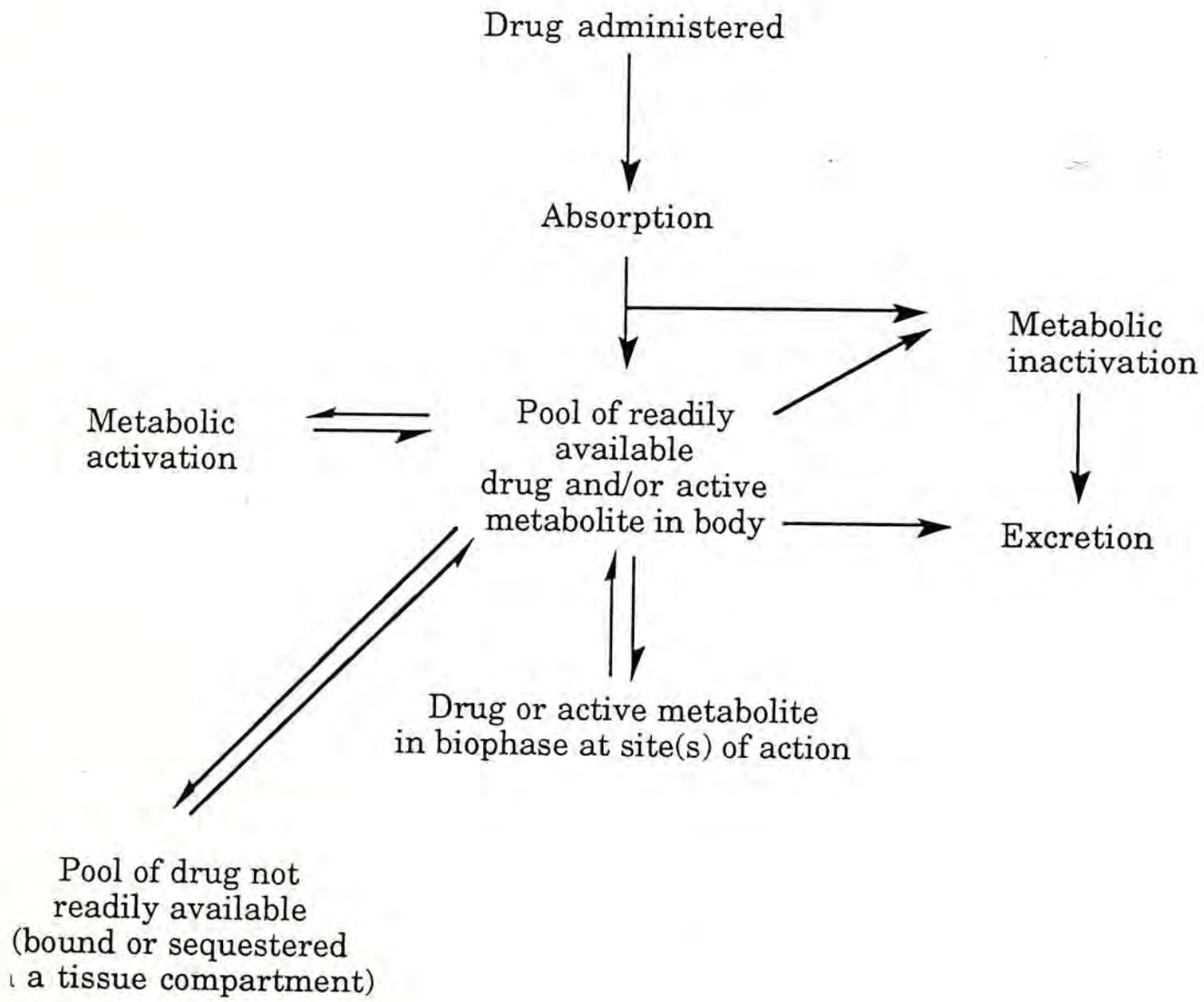
Pharmacokinetics, a discipline that concerns the study and characterization of the time course of drug levels in the body after administration. It is best defined as the study of the rates of the transfer processes associated with the absorption, distribution, metabolism and elimination of a drug in the body (Fig 2).

The administration of a bolus dose intravenously is to introduce the drug as one dose directly into the blood stream in a relatively short time. Usually extensive dilution occurs within a short time of injection, and mixing with blood is essentially complete within 10 minutes. While being transported in the blood stream the drug rapidly diffuses into the surrounding tissues as it passes in through capillary beds. Decline of the drug in the blood continues as it passes into highly perfused organs and then out of these into less perfused tissues. The blood concentration of the drug equilibrates rapidly with that in various tissues until a distribution equilibrium is obtained. The phase prior to attainment of distribution equilibrium is commonly refered to as distribution phase. The subsequent phase, when the decline in blood reflects a proportional loss of drug from the body, is the elimination phase.

Pharmacokinetics uses the techniques of compartmental analysis (the body is assumed to consist of different compartments) in explaining the parameters obtained from plasma or urinary concentration profiles of drugs. This approach involves the adoption of the simplest mathematical model which is composed of the least number of compartments required to interpret the data. In the simpler of the two models, the drug is assumed to distribute

instantaneouly into a space called the central compartment. Then the drug is simultaneously distributed into a second space and eliminated. The three-compartment model assumes that there are two distinct spaces to which the drug distributes from the central compartment at measurable different rates.

Fig 2. An outline of the processes with which pharmacokinetics is concerned.

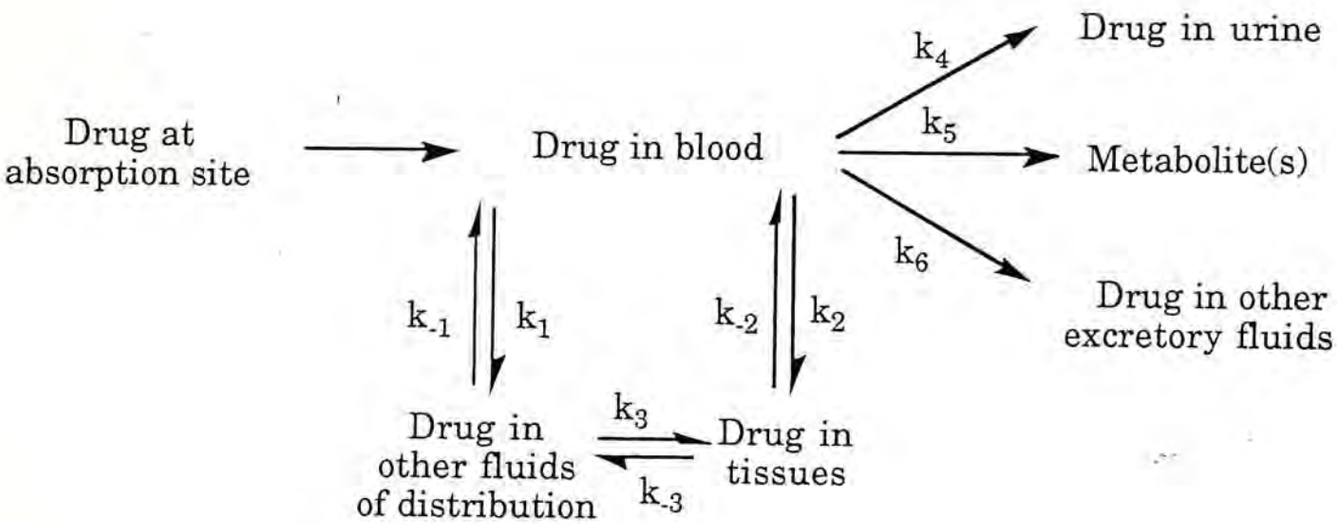


1.3.1. Distribution and Elimination

The transfer of drug from its absorption site to the blood stream is complete within a relative short time. While being transported in the blood stream the drug rapidly diffuses into the surrounding tissues as it passes through capillary networks. Since most drugs are sufficiently lipid soluble to cross cell membranes and distribute in the intracellular fluids of various tissues, the body can be assumed to consist of several separate compartments and each containing some fraction of the administered dose. The transfer of drug from one compartment to another (Fig 3) is associated with a rate constant (k) and this process is called distribution. Distribution is usually a rapid and reversible process. The transfer of drug from the blood to the urine or other excretory compartments and the enzymatic or biochemical transformation (metabolism) of drug, are usually irreversible processes, termed as elimination. Elimination is responsible for the physical and biochemical removal of drug from the body.

Distribution and elimination occur simultaneously, but the rate of distribution is often faster than the elimination. Therefore drugs usually attain distribution equilibrium before a measurable fraction of the dose is eliminated. In such circumstances, the body appears to have the characteristics of a single compartment. Unfortunately this simplification can not be applied to all drugs. In some cases, a considerable fraction of the drug dose is eliminated before the distribution equilibrium is reached. Such drugs exhibit the behaviours of a multicompartment system within the body.

Fig 3. Schematic representation of drug absorption, distribution and elimination.



1.3.2. Non-compartmental analysis

The calculations involved in the multicompartmental methods 0f analysis (mentioned in Section 1.3.) are complicated and therefore non-compartmental or one compartmental methods have been widely used nowadays. It assumes the drug does not exhibit the distribution phase (no compartment) but is homogenously distributed throughout tissues and fluids of the body. The analyses are based on the theory of statistical moments (1-2). The two most important primary pharmacokinetic parameters, clearance and volume of distribution at steady state, can be easily obtained by these methods.

Clearance (Clp) is defined as the volume of plasma cleared of drug per unit time. We can refer to Clp by a particular organ, metabolic pathway or

by the whole body. Total body Clp is the sum of all different Clp processes occurring for a given drug. Clp determines the maintenance dose rate required to achieve a desired plasma concentration or drug effect at steady state. We can use Eq. 1 to obtain the Clp of a drug from the steady state drug concentration during a constant intravenous infusing.

Clp = dose rate/steady state blood concentration . . . . . [1]

Alternatively, for a single dose, we can take frequent blood samples and measure the drug concentration and calculate the area under the drug concentration versus time curve (AUC). The Clp can then be obtained by means of Eq. 2.

Clp = dose/AUC . . . . . [2]

The volume of distribution of a drug at steady state (Vss) rarely corresponds to the anatomic volume such as the volume of total body fluids but represents the proportionality constant relating the amount of drug in the body at steady state after prolonged constant rate of intravenous infusion, or repetitive administration or average drug concentration at that time. Vss depends on the drug's physicochemical properties and patient characteristics like body weight and composition. It is a valuable parameter that can allow one to determine or predict the distribution of drug and to compare the distribution characteristics of various drugs. Estimation of Vss does not require data obtained at steady state; this distribution parameter can be calculated after the administration of single dose of a drug (3-4) Eq. 3 and 4.

$$Vss = dose(AUMC)/(AUC)^2 \ldots\ldots [3]$$

$$Vss = Clp \times MRT \ldots\ldots [4]$$

where AUMC is the total area under the first moment curve and MRT (mean residence time) is governed by

$$MRT = AUMC/AUC \ldots\ldots [5]$$

Biological or elimination half-life ($t_{1/2}$) is defined as the time required for the reduction to one-half of the initial drug concentration or activity after the drug has been absorbed and has reached a desirable level of activity. Its value can be readily determined for most drugs by administering a dose of the drug to a subject. Blood samples are then taken at various time intervals and followed by assay for drug content. The value is equal to the slope of the curve when the logarithmic concentrations are plotted against time on semilogarithmic graph paper. The elimination half-life is a unique property of a given drug and hence it is an important parameter which can help to establish rational regimes for drugs, eg. initial doses, maintenance doses or doses for sustained action dosage forms. The $t_{1/2}$ is also a composite pharmacokinetic parameter determined by both Clp and Vss according to Eq. 5.

$$t_{1/2} = \ln 2\,(Vss)/Clp \ldots\ldots [5]$$

The elimination of a drug from the body is usually a result of metabolic and excretion processes. The rate of elimination for most drugs is usually described by a first-order kinetic and the elimination rate constant (ke) for a first-order process is inversely proportional to $t_{1/2}$ (Eq. 6).

$$ke = \ln 2/(t_{1/2}) \quad \ldots\ldots [6]$$

Like $t_{1/2}$, ke is the other secondary pharmacokinetic parameter (derived from primary parameters) that determines the action of the body when it is disposing of a drug.

1.4. PHYSICOCHEMICAL PROPERTIES OF DRUGS

Although the passage of drugs through biological membranes also involves aqueous diffusion and carrier-facilitated transport, in most circumstances, absorption of drugs from the site of administration into the blood circulation, its distribution, excretion and renal tubular reabsorption are mainly achieved by passive lipid diffusion of nonionized drug molecules across biological membranes. The cells of the membrane barriers can be described as a lipid phase separating two aqueous phases. Lipid soluble substances are taken up at one interphase according to their lipid/water partition coefficient, diffuse across the lipid phase according to their concentration gradient, and are redistributed in accord with their lipid/water partition coefficient at the opposite interphase. It is assumed that lipid cell membranes are impermeable to the ionized form of a weak electrolyte drug, but that the nonionized form can equilibrate freely across the membranes. Therefore these membranes act like lipoid barriers which restrict the movements of the molecules, and the rate of lipid diffusion of drug molecules across membranes is represented by the Fick's law which in turn is governed by the physicochemical properties of the drug, such as the degree of ionization, lipid solubility and protein binding ability. Factors that favour rapid diffusion are a low degree of ionization of weak electrolyte drug, a high lipid-soluble nonionized form at physiological pH and an absence of protein binding. These physicochemical properties can be measured with ease by simple laboratory procedures. The obtained values will be useful in determining the relative penetration power of a series of drugs across

membranes and one can also relate these values to interpret the corresponding pharmacologic activities and pharmacokinetics of drugs.

Removal of an electron

1.4.1. Ionization

The molecular structures of many drugs contain groups ionizable in aqueous solutions to anionic or cationic forms, which may be quantitatively expressed by its acidic ionization constant (Ka). This dissociation exponent, represented by pKa, is the negative logarithm of the dissociation or ionization constant. Since only the nonionized molecules are lipid soluble and hence the ability of such drugs to pass through lipid membranes depends on the proportion of nonionized molecules. A knowledge of the pKa is necessary in establishing whether the drug is predominantly in the nonionized form in the biological fluids. The degree of ionization is dependent on the ionization constant (Ka) and the pH of the aqueous solution which can be readily illustrated by following equations derived from the Henderson-Hasselbalch Equation (Eq. 7).

$$\% \text{ ionization for an acid} = 100/[1+\text{antilog}(pKa-pH)] \quad [7]$$

$$\% \text{ ionization for a base} = 100/[1+\text{antilog}(pH-pKa)] \quad [8]$$

Hence the higher the pH of the medium, the higher is the ionization of acidic drugs and the lower is the ionization of basic drugs. When the pH values of the aqueous phases on either sides of a membrane are different, acidic drugs will be in a higher concentration on the more alkaline side whereas basic drugs will be in a higher concentration on the more acidic side. The ratio of

the concentration (R) of a drug on either side of a membrane is thus proportional to the tendency of the drug to ionize and to the difference in pH across the membrane. General equations to describe this concentration ratio of acidic and basic drugs are illustrated as follows:

For acidic drugs, $R = (1+10\ pH_1\text{-}pKa)/(1+10\ pH_2\text{-}pKa)$ . . . . . [9]

For basic drugs, $R = (1+10\ pKa\text{-}pH_1)/(1+10\ pKa\text{-}pH_2)$ . . . . . [10]

where pH_1 and pH_2 are the values on either sides of the membrane.

A number of methods have been available to determine the ionization constants. Potentiometric titration is by far the most convenient and usually enables the Ka to be determined in a short time. Although ultraviolet/visible spectrophotometry is a time consuming method and can only be used for substances which absorb ultraviolet or visible light, it is particular suitable for sparingly soluble substances and can also work at either very low or high pH values which are beyond the range of potentiometry. Conductimetry is another possible alternative and is useful for very weak acids. Like conductimetry, solubilities determination is not so accurate a method, but it is adequate in the cases where a substance is too insoluble in water for potentiometry or conductimetry, and has no obvious ultraviolet absorption.

1.4.2. Partition coefficient

It is known that the lipid solubility of the unionized forms plays a

major role in determining the rate of drug diffusion and absorption. The term "lipid solubility" is often used to characterize the ratio or partition coefficient (K) between absolute solubility in a lipid phase and in aqueous phase. Since the investigation by Collander and Barlund (5) on the significance of lipid solubility for the penetration of drugs through biological membranes, various physicochemical model systems have been sought to reflect the *in vivo* drug penetration. However the measurement of lipid solubility of drugs with respect to a biological membrane *in vivo* is rather difficult. A reasonable experimental estimate of this factor is by measuring the lipid/water partition coefficient of the unionized form. Buffer or saline solution at pH 7.4 is presumably used as the aqueous phase so as to simulate physiological conditions as far as possible. Even within the same compound, different lipid/water systems give different K values and therefore the applicability to the biological system of K obtained in this manner will surely depend on the nature of organic solvent used to represent the lipid phase, i.e., how closely its characteristics resemble the complicated lipoprotein structures of a cell membrane. Therefore when considering the lipid solubility of a series of compounds, it is essential to measure K in several different solvents-buffer system in order to find trends which can be expected to apply to membrane structures. Some workers (6-7) have shown that the K value of n-heptane/water can be used as a reliable criterion for the ability of substances to diffuse into the central nervous system. Good correlations with the rates of passage across biological membranes are also obtained with solvents like dichloromethane and octanol. The absolute values of K do not proven useful,

rather, it is the rank order in a series of compounds that can be used to reflect the rates of penetration. In general, the higher the partition coefficient the higher is the affinity for lipid membranes and the more rapidly the drug passes through the membranes. Endogenous end products (metabolites) usually have relatively lower K values than their parent drugs because of the additional hydrophilic group(s).

1.4.3. Plasma Protein Binding

Plasma proteins are composed of amino acid with polar and nonpolar side chains that protrude into the surrounding which are largely responsible for drug binding. This type of binding is reversible and the magnitude is dependent on the concentrations of both proteins and drugs in the plasma. The affinity of drug to proteins is governed by its chemical and physical properties. The forces involved in drug binding to protein are primarily hydrogen and hydrophobic bonds although ionic interaction and van der Waals forces may contribute. The presence of protein binding can reduce the passive diffusion firstly because the total free drug concentration is decreased which cause a drop in concentration gradient across the membrane and secondly the bound drug is not freely diffusible through membrane due to its enormous size. Protein bindings can be altered by a variety of pathological conditions such as hepatic insufficiency (8) and hypoalbuminemia (9). This consequent alteration of binding could have varying effects on the pharmacokinetic parameters (10-11).

The simplest type of binding can be expressed as a reversible

reaction:

$$K = [D][P]/[DP] \ldots\ldots [11]$$

where K = equilibrium dissociation constant, [D] = drug concentration, [P] = protein concentration and [PD] is the drug-protein complex concentration. Since the total concentration of protein [Pt] equals the sum of the concentrations of free protein ([P]) and protein engaged in binding ([DP]), the term [Pt] - [PD] can be substituted for [P] in Eq. 11:

$$K = [D]([Pt] - [PD])/[PD] \ldots\ldots [12]$$

which may be rearranged to the form:

$$r = [D]/(K + [D]) \ldots\ldots [13]$$

where the ratio [PD]/[Pt] is represented by r. If there is a number (n) of identical separate binding sites, there are a series of equations of the type given in equation 13, but these may be summed as:

$$r_1 + r_2 + r_3 + \ldots + r_n = r\text{total} = n[D]/(K + [D]) \ldots\ldots [14]$$

The values of [D] and r can be measured experimentally, hence the values of n and K can be determined from the appropriate graphical plots. The most commonly used procedure for the analysis of data on protein-binding is the Scatchard plot of r/[D] against r. The equation, obtained by rearranging Eq. 14 is given by:

$$\frac{r}{[D]} = \frac{n}{K} - \frac{r}{K} \quad \ldots\ldots [15]$$

Scatchard in 1949 has shown that straight line plots are obtained from analysis of binding data for various inorganic ions and for some drugs, but with many drugs and endogenous metabolites the plots depart substantially from linearity. A curvilinear Scatchard plot indicates that there is more than one type of binding site and that the different types differ in their dissociation constants for the binding.

A number of methods have been applied to the study of drug-protein binding interaction. Equilibrium dialysis is often regarded as the standard method which is based on the principle that unbound drug will equilibrate across a semipermeable membrane between a protein solution containing bound drug and a protein-free (buffer) solution. At equilibrium the drug concentration in the buffer compartment is considered to represent the unbound drug concentration in the original sample (12). The convenience and simplicity of the method make the technique well suited for the determinations involving large numbers of samples although nonspecific membrane binding and sample dilution may hamper interpretation of free drug values. Ultrafiltration, a new and promising procedure, is often used by means of a fine-mesh membrane filter to isolate unbound from protein-bound drug. Its major advantages are speed and the absence of dilutional effects seen in equilibrium dialysis. Nonspecific membrane binding and protein leakage are the generally recognized limitations which could produce unreliable and erroneous results (13). Ultracentrification is a technique to

separate fractions of a plasma sample into concentration gradients relied on relative size, shape and molecular weight of the particles in the centrifuge tubes. The goal of this method is the absence of nonspecific binding because a membrane is not used. The common disadvantages are slow and extremely expensive. In summary each separation method has been observed to produce some bias, which complicates the comparison of results between different methods. Until recently no method has been considered sufficiently reliable and convenient to apply directly to the study. Therefore the choice of method involved in binding study is very important. It always depends on the nature of the bound drug, available sample size, number of studied samples, time and cost of the experimental work.

1.5. GAS-LIQUID CHROMATOGRAPHY

1.5.1. Introduction

Chromatography embraces a variety of processes which are based on differential distributions of the sample components between two phases. One phase remains fixed in the system and is called stationary phases. The another phase, called mobile phase, percolates through the interstices, or over the surface, of the fixed phase. The movement of the mobile phase causes a differential migration of the sample components. The migration rates of various components depend on their physical and chemical properties, thus chromatography provides an important analytical tool in ressolving the components from a sample mixture.

A gas-liquid chromatographic system essentially consists of a carrier gas, an injector port, a column, a detector and a signal analyzer. The carrier gas, supplied from a pressurized tank, passes through one or more pressure regulators which control the flow rate through the apparatus. The sample is introduced into a heated chamber either through a silicone rubber septum with hypodermic syringe if it is a liquid, or by means of a special sampling valve if it is a gas. From here the carrier gas carries the sample components through the column where they are separated accordingly and one after the other pass through a detector which sends a signal to a recorder or integrator. A thermostatted oven is provided for the column, injector and detector.

1.5.2. Quality control in chromatography

The analysis of therapeutic agents offers problems that challenge the

best analytical techniques and instrumentation available. The measurement of low concentration of therapeutic agents and their metabolic or degradative products in biological fluids is particularly difficult. The compounds are usually found in minute quantities and other compounds present may cause analytical interferences. Most measurement techniques are not selective enough to measure individual components in a sample mixture. In many cases it is necessary to separate the desired compound from the others. Good separation reduces selectivity problems and permits the use of maximum detector sensitivity with minimum background interferences. The development of chromatography (gas-liquid chromatography, GC and high performance liquid chromatography, HPLC) has tackled this analytical task and its application in the analysis of exogenous substances in biological fluids has passed from the realm of the experimental to a routine procedure for everyday chemical and clinical analysis. One of the most important features in the development of the modern chromatographic techniques is to acquire a high-quality of quantitative analysis. Therefore laboratories, either in academic or industrial firms, which employed chromatography must take appropriate and considerable measures to institute and maintain an adequate quality control. In most circumstances, it is of great value to have the ability for accurate and rapid quantitative analysis. This ability is usually depending on the attention to details given by the chromatographer as to the design of the equipment. A careful calibration will certainly minimize the effect of unavoidable variations in instrumentation and procedures, and from different operators and laboratories. Also, careful attention must be given to

all preparation stages of the analysis from sample collection to the final calculation of the results.

Quality control of the assay must start long before the sample is actually analyzed. In summary, meticulous adherence to properly established operational procedures as well as the use of proper controls and internal and recovery standards are necessary if any meaningful approach to quantitative analysis is to be made. In determining whether a substance should ever be examined for quantitation in a particular biological sample, special attention must be paid to the following important items. With reasonable attention to these details, chromatography with appropriate detection means can be regarded as a routine, highly accurate, sensitive and reproducible analytical technique (14).

1.5.2.1. Sampling

Since a non-representative sample is of no value however carefully it has been analyzed, no result of a determination can be of any meaning unless the material submitted for analysis is a statistically valid, representative sample of what is to be ~~measured~~ analyzed. This of itself can be a complicated problem (15). Any portion of a liquid preparation may be considered as representing the whole, provided the liquid has been thoroughly mixed immediately before the sample is taken; e.g., an aliquot is sampled from a bulk urine sample is thought to be representative. Similarity in the production of pharmaceutical products like tablets, there is seldom a reason to believe that any one tablet is more representative than any other. In all sample collection procedures it is

recommended that several sub-samples are taken from each product or bulk sample. These should be processed individually and the results compared. Any significant variation of the results will immediately indicate whether or not the procedure adopted was satisfactory.

1.5.2.2. Storage

Samples may be perishable and subject to decomposition under certain experimental conditions (enzymatic or bacterial degradation may occur). Sometimes it may be better to extract and then store the analyte in the sample rather than to store the whole sample directly. Hence the method and stability of the storage are very important. Preliminary tests should be performed to investigate the stability of the analyte(s) in different sample media such as serum, whole blood and urine under various conditions. In general, samples of various types are kept from direct sunlight and stored in a freezer at low temperature (usually at -20°C).

1.5.2.3. Extraction and clean-up procedures

Homogenation of the sample in a blender, automatic shaker or by ultrasonic disintegration may be necessary before extraction. Extraction is an important step, especially for biological fluids, to isolate the desired substance (and its metabolites) from endogenous materials so that these substances are in pure enough form to allow detection and determination without serious interference. A large variety of solvents or solvent mixtures are available for liquid-liquid extraction. If a biological substance has metabolites, then the

extraction efficiency must be high for both the parent compound and its major metabolites. The pertinent up-to-date literature should be consulted for specific substances. Buffering of the material at one or more pH values may be necessary to optimize extraction with organic solvents or resin columns (16). For more polar chemicals which can not be extracted by those organic solvents or the recovery is not satisfactory, it is also possible to conduct a solid phase extraction (using disposable cartridge, switching column or pre-column in HPLC).

Other clean-up procedures involved back extraction (acid or base wash), ultrafiltration, centrifugation and deproteinization of plasma followed by filtration are the commonly applied methods before the injection of analyte(s) into the chromatography.

1.5.2.4. Recovery

A Recovery study is often performed by adding recovery standards to the samples before extraction. Losses may occur at each step in the operation and therefore the overall recovery for the whole procedure is important. In general, this should not fall below 70% if the method is considered to be satisfactory. It may be desirable to fortify (or spike) samples with a series of increasing amounts of the recovery standard to develop a standard recovery curve. Ideally, recoveries should be determined at several concentrations over the range in which results are being reported. But it is still useful if the recoveries are determined either at both the maximum and minimum concentrations or at a particular concentration within the calibration curve.

These standards can be used to estimate the proportion of analyte actually extracted from biological fluids. They may also be added to the samples or to known controls run with the analysis.

1.5.2.5. Internal standards

For accurate quantitation analysis of liquid samples, internal standards are desirable provided they do not create difficulties in sample preparation. An internal standard is a maker compound which is added in accurately known concentration to the sample. It may be added in the last extraction or to the sample before clean-up and extraction. They should be a substance, not expected to be present in the biological fluid examined, which is compatible with the sample and differs in retention time from the substances to be measured so that its peak is separate from all the other peaks. It provides a scale value for quantitation. Ratios of the analytical peaks (either peak heights or peak areas) to that of the internal standard permit determination of concentration ratios. As with the recovery standards, a standard curve for various internal standards may be prepared in the range of the sample concentrations to be determined. Such curves may be compensate for non-linear detector response (17).

1.5.2.6. Derivatization

Since quantitation requires good peak separation, it may be necessary to treat the sample to permit peak separation if several substances in the sample with closely related peaks are present or the substance itself is not a

good detectant, e.g., poor ultraviolet and fluorescence absorption properties in HPLC, or non-volatile enough to be detected by GC. Derivatization may also permit separation due to changes in column adsorption and consequent changes in retention times for the various substances. The formation of derivatives usually increase the selectivity as well as the sensitivity of the method and may allow the measurement of nano- or picogram quantities of substances. Some commonly used derivatization techniques are acetylation, methylation, chemical reduction and halogenation. For most quantitative purposes derivatization is carried out in the final extraction stage prior to evaporation. On-column derivatization is used primarily for tentative identification by 'peak shifts' rather than for quantitation (18-19).

1.5.2.7. Calibration standards

Pure, properly characterized standards at various concentrations should be used frequently to calibrate the instrument. A calibration curve is necessary to allow accurate quantitation as well as to determine the linear portion of the operating curve for the detector and the point where detector overload begins.

1.5.2.8. Controls

As in every other chemical analysis, known accurate controls should be run with the samples to verify that each step in the overall procedure is under control. The controls should be in the same concentration range as the substances to be measured.

1.5.2.9. Quality control in the experimental set up

The above mentioned preparative stages and treatments are the essential and key elements to establish a good laboratory practice (GLP). In order to achieve an accurate and reproducible procedure, we have to not only adequately followed these working steps as the routine analysis control, but also pay considerable attention in the relevant factors during the course of the experimental work, such as precise evaluation of column status and rigid maintenace of the injector ports, analytical columns gas flow rates and detector. If we believe that our developed chromatographic methods are reliable and sensitive enough in the determination of drugs in biofluids and these methods will work equally well if someone uses the methods in other laboratories.

CHAPTER 2

A GAS-LIQUID CHROMATOGRAPHIC METHOD FOR STUDYING THE EFFECT OF ADRENALINE ON VENOUS PLASMA CONCENTRATIONS OF BUPIVACAINE AFTER INTERPLEURAL ADMINISTRATION

2.1. INTRODUCTION

Bupivacaine (Marcain®), an anilide type of local anaesthestics with high potency and long duration of action, is recently used to provide post-operative analgesia, via interpleural administration for patients who had undergone abdominal, thoracic and renal operation (20). The adverse effects of bupivacaine are correlated with its concentrations in the systemic circulation and therefore studies of the plasma bupivacaine concentrations are often performed to determine the relative risk of systemic toxicity in order to make its use safer for patients.

Several methods for the determination of bupivacaine in plasma have been reported recently, using GC (21-27), GC-mass spectrometry (28) or HPLC (29-33). In all these methods, bupivacaine was initially isolated from biological fluids via solvent extraction before chromatography. A single-step extraction with n-hexane (22) , ether (23) or dichloromethane (24) was employed for the determination of bupivacaine using capillary gas chromatography.

Although nowadays capillary GC is established as a routine technique, yet the demand for GC methods employing packed columns is still

great because packed columns are more economical and they have higher sample capacity and are not easily overloaded. Unfortunately, when packed columns were used for the determination of bupivacaine, the extraction procedure became more tedious. For instances, the drug was first extracted at alkaline pH into diethyl ether, back extracted into 1M HCl and finally extracted again at an alkaline pH into ether (24); or the plasma in 0.1M H_2SO_4 was washed with dichloromethane and then made alkaline with ammonium buffer followed by dichloromethane extraction (25). Other sample treatment such as precipitation of proteins with trichloroacetic acid (26) was also required. Chan (27) has developed a single-step extraction with a diethyl ether and dichloromethane mixture in the ratio of 3:1. However, the solvent mixture could only recover about 70% of bupivacaine from plasma under alkaline conditions. We describe here an accurate, rapid and selective GC method to determine bupivacaine in human plasma. The procedure was based on a single-step extraction of the drug from basified plasma into n-hexane.

The position of interpleural analgesia in modern anaesthetic practice is under review and many questions remain to be answered (34). The successful dose of bupivacaine has varied from 0.5% bupivacaine 8 ml (35) for pancreatic cancer pain, to 0.5% bupivacaine 30 ml (36) for cholecystectomy and even 0.75% bupivacaine 60 ml has been used in bilateral block (37). As with intercostal block (38), interpleural bupivacaine is absorbed rapidly (36,39-41) and concern regarding toxic concentrations of bupivacaine led to the routine addition of adrenaline (vasoconstrictor) to the local anaesthetic solution. However, there have been no controlled studies to confirm that this

decreased the absorption of bupivacaine. The use of adrenaline has been criticized because significant cardiovascular side effects have been reported when infusions have been used (42). A recent study of interpleural bupivacaine, with and without adrenaline, administered a fixed volume and dose of local anaesthetic but did not analyse the effect of patients' body weight and size on the peak concentrations of bupivacaine (43). The time to peak absorption was much later in their adrenaline group compared with the previous reports while, in the control group, results were skewed by one patient who had values three times that of the other patients.

The aim of this study was to apply the GC method described here to determine the plasma concentrations of bupivacaine after the intrapleural administration of a body-weight related dose to patients and to examine the effects of added adrenaline 1:200,000 (5 μg/ml) on the absorption of interpleural bupivacaine.

2.2. EXPERIMENTAL

2.2.1. Reagent and apparatus

Bupivacaine and etidocaine (internal standard) hydrochlorides were supplied as gift by Astra Pharmaceutical Production AB (Sweden). Glass-distilled AnalaR-grade n-hexane and methanol were purchased from E. Merck (Darmstadt, FRG). The following glassware was used: 15-ml capacity centrifuge tubes with well fitted screw caps containing PTFE linings, and 15-ml capacity Quick-fit glass tubes with tapered base of 50 µl. All glassware was cleaned and silanized with 3% hexamethyldisilazane (HMDS) in chloroform before use to avoid possible loss due to adsorption onto the glass wall.

2.2.2. Gas chromatograph

A Varian Model 6000 gas chromatograph (Varian Association, Palo Alto, CA, USA) equipped with a nitrogen-phosphorus detector was used for analysis. The coiled glass column (2m x 2mm i.d., 6mm o.d.) was packed with 3% W/W SP2250 material on Chromosorb W, 80-100 mesh. The column temperature was maintained at 240°C and that of the injector and detector at 300°C. The gas flow rates were: nitrogen (carrier gas) at 30 ml/min; air at 175 ml/min and hydrogen at 4.5 ml/min. The signal was recorded and displayed by a flat-bed chart recorder (Linseis, Model L6512).

2.2.3. Standard solutions and recovery test

Stock solutions containing 1 mg/ml of bupivacaine and etidocaine hydrochlorides were prepared separately in methanol and stored at -20°C until use. Working standard solutions with concentration of 50 μg/ml were freshly prepared when required by appropriate dilution of the respective stock solutions with methanol.

Drug plasma standards were prepared by spiking blank control plasma (0.5 ml) with appropriate microlitre volumes of working standard drug solution to obtain seven calibration standards with the following concentrations of bupivacaine: 0.1, 0.2, 0.5, 1.0, 1.5, 2.0 and 4.0 μg/ml, and an aliquot of the etidocaine (8 μl) working standard solution was added to each solution as the internal standard.

To assess the recovery of bupivacaine from plasma samples by the extraction procedure, the drug was added to drug-free plasma at 2.0 μg/ml and assayed with the internal standard (often referred as marker by some authors) as described. For comparison, the same concentration of bupivacaine and marker were prepared in methanol, evaporated and assayed with the extraction step omitted. The corresponding peak height ratios were then compared.

2.2.4. General analytical procedures

To a plasma sample (0.5 ml) in a 15 ml centrifuged tube, 8 μl of the

internal standard solution was added, followed by 30 µl of 5M sodium hydroxide solution. The extracting organic solvent (5 ml n-hexane) was added. After mixing thoroughly with the aid of an automatic shaker (10 min at 30 r.p.m.), the organic extract was separated by centrifugation and then evaporated to dryness at 45°C under a gentle stream of nitrogen in a 15-ml evaporating tube. The residue was redissolved in 10 µl of methanol and 1 µl aliquot was injected into the GC for analysis.

2.2.5. Data analysis

Plasma concentration data were analysed by a model-independent method (non-compartmental method) based on statistical moment theory (1-2,44). The principle of non-compartmental method has been briefly outlined in Chapter 1. The analysis was carried out using a microcomputer program PKCALC (45) and the augmented ESTRIP (45) which determined the elimination half-life according to the terminal linear position (usually the last four to six points) of the plasma concentration-time curve. The computer calculated the linear least squares regression line associated with the data subset. Area under the plasma concentration-time curve (AUC), apparent volume of distribution (Vss) and total body clearance (Clp) were computed by PKCALC. The Students unpaired t-test was used for comparisons where appropriate. $p \leq 0.05$ was accepted as statistically significant.

2.2.6. Post-operative analgesia procedures for patients

After approval from the Research Ethics Committee of the Faculty of Medicine, The Chinese University of Hong Kong, twelve Chinese patients for elective cholecystectomy gave informed consent for this study. Patients were not currently taking any medications before the operation and had no history of pulmonary disease or allergy to local anaesthetics. No patient admitted to regular alcohol intake or smoking history. All patients had normal routine haemotology and biochemistry, including liver function ~~tests~~.

Anaesthesia was induced with thiopentone 4 mg/kg, fentanyl 2 mg/kg and atracurium 0.5 mg/kg. An interpleural catheter (16-gauge extradural) was inserted at the end of the operation, before antagonism of neuromuscular block with neostigmine and atropine. Patients were allocated randomly to receive bupivacaine 2.5 mg/kg with or without the inclusion of 1:200,000 adrenaline as available commercially from Astra Pharmaceuticals. This was injected over 2 min with the patient lying supine. Further interpleural injections were not performed. If the patient was still in pain after 30 min, morphine in 2.5 mg was administered intraveneously until the patient was feeling comfortable. Pethidine was given via intramuscular route for additional post-operative analgesia when required by patient. Duration of interpleural analgesia was taken as the time from interpleural injection to the time that patients first requested pethidine.

Patients were questioned directly about severity of pain on a simple scale from 0 to 3, with 0 representing no pain, 1 slight pain, 2 moderate pain and 3 severe pain. Assessment of pain score was repeated at 5, 10, 15, 20, 30,

45, 60, 120, 240, and 480 min after bupivacaine was administered and blood samples were obtained before and at the above time intervals. The collected samples were centrifugated to yield plasma and immediately stored at -20°C in a refrigerator until analysis.

2.3. RESULTS

2.3.1. Choice of extraction medium and internal standard

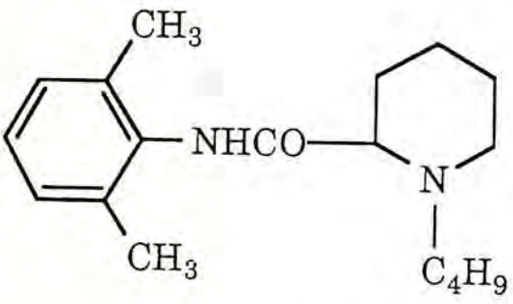
Diethyl ether was commonly used to extract drugs from pharmaceutical preparations and biological fluids. This solvent usually gives reasonable high extraction recovery and is easy to remove at room temperature owing to its high volatility property. In our preliminary study, however, we have found that some endogenous plasma materials were extracted with bupivacaine, resulting in interfering peaks overlapping with the analytical peaks in gas chromatography when packed columns were used. The suitability as the extraction medium of several organic solvents, including chloroform, dichloromethane, dichloroethane, n-hexane and a mixture of ether and dichloromethane (3:1/v:v) was therefore examined (Table 2). It was shown that chloroform, diethyl ether and n-hexane gave a good recovery (>85%) however, chloroform, like diethyl ether, was not suitable to use as an extraction medium because interfering substances were present in the extract. Although interfering peaks were not detected in the case of dichloroethane, the corresponding recovery was relatively poor (about 60%) and therefore n-hexane was chosen as the organic extract in the study. Etidocaine was selected as the internal standard because its chemical structure and properties such as lipophilicity are similar to those of bupivacaine (Fig 4).

Table 2.

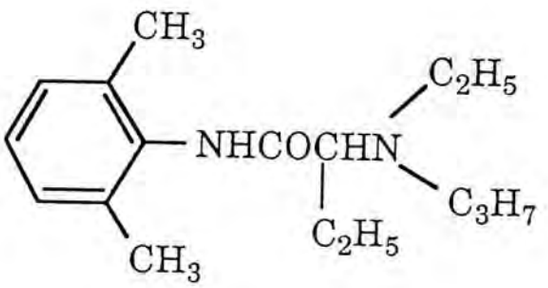
Comparison of various organic solvents for the extraction of bupivacaine.

Solvent	% recovery (n=6) Mean ± S.D.	Comment
Chloroform	85.6±1.9	analytical peaks were interfered
Dichloromethane	48.6±2.5	same as above
Diethyl ether	95.8±3.3	same as above
Ether/CH_2Cl_2	52.7±4.5	same as above
Dichloroethane	61.4±2.2	no interferring peaks observed
n-Hexane	91.9±2.8	same as above

Fig 4. Chemical structures of bupivacaine and etidocaine (internal standard).



Bupivacaine



Etidocaine

What about extraction of etidocaine?

2.3.2. Gas chromatograph

Typical chromatograms of the extracts from blank plasma and from plasma of patient during interpleural analgesia obtained using the procedure described above are shown in Fig 5. The anlytical peaks of both internal standard and drug were well resolved with good symmetry and their retention times were 3.0 and 7.2 min respectively. No interfering peaks which masked the analytical peaks were observed from the blank and patient's plasma samples.

As illustrated by Table 3, the performance of the GC system was considered satisfactory for the assay of bupivacaine. The calibration graph (Fig 6) was linear over the range of 0.1 to 4.0 μg/ml. The least-squares linear regression line which related the best fit peak height ratios (y) and bupivacaine concentrations in μg/ml (x) could be described by an equation of y = 0.996x+0.0190 and the correlation coefficient was 0.9994. The relative standard deviation (RSD) for the determination of bupivacaine varied from 2.0 to 5.8% within the linear calibration range. The RSD for the day-to-day variation using a standard at 2.0 μg/ml (n=10) was 6.9% and the lowest detection limit of the assay was 0.01 μg/ml when a 0.5 ml plasma sample was used.

2.3.3. Patients study

There were no ~~detected~~ Significant variation with age, weight or total anaesthetic

Fig 5. **Chromatograms obtained from: (left) blank plasma extract and (right) extract of plasma of patient containing 2.8 μg/ml of bupivacaine. Chromatographic conditions were as described above. Peaks: 1=bupivacaine and 2=etidocaine (internal standard).**

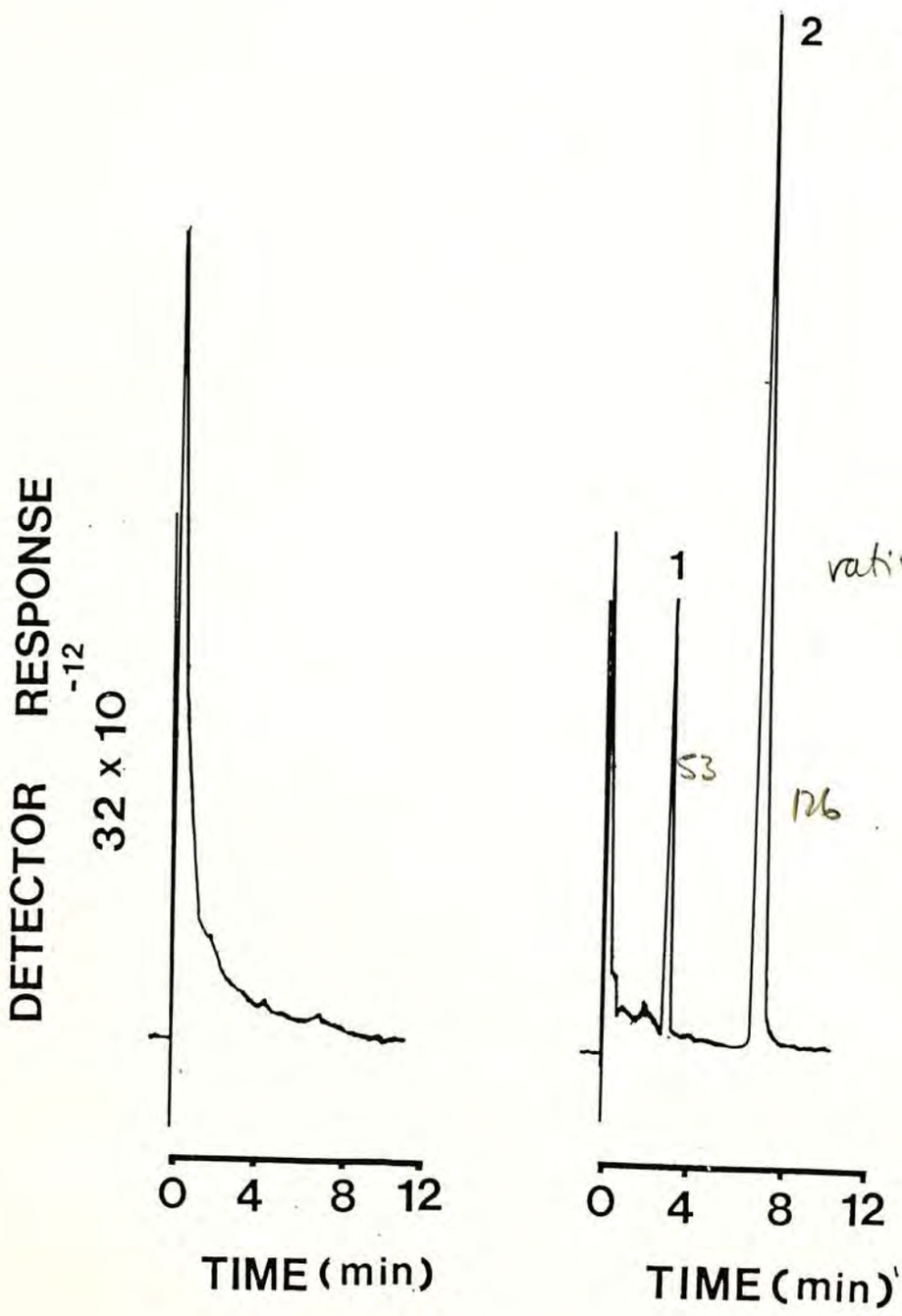


Table 3.

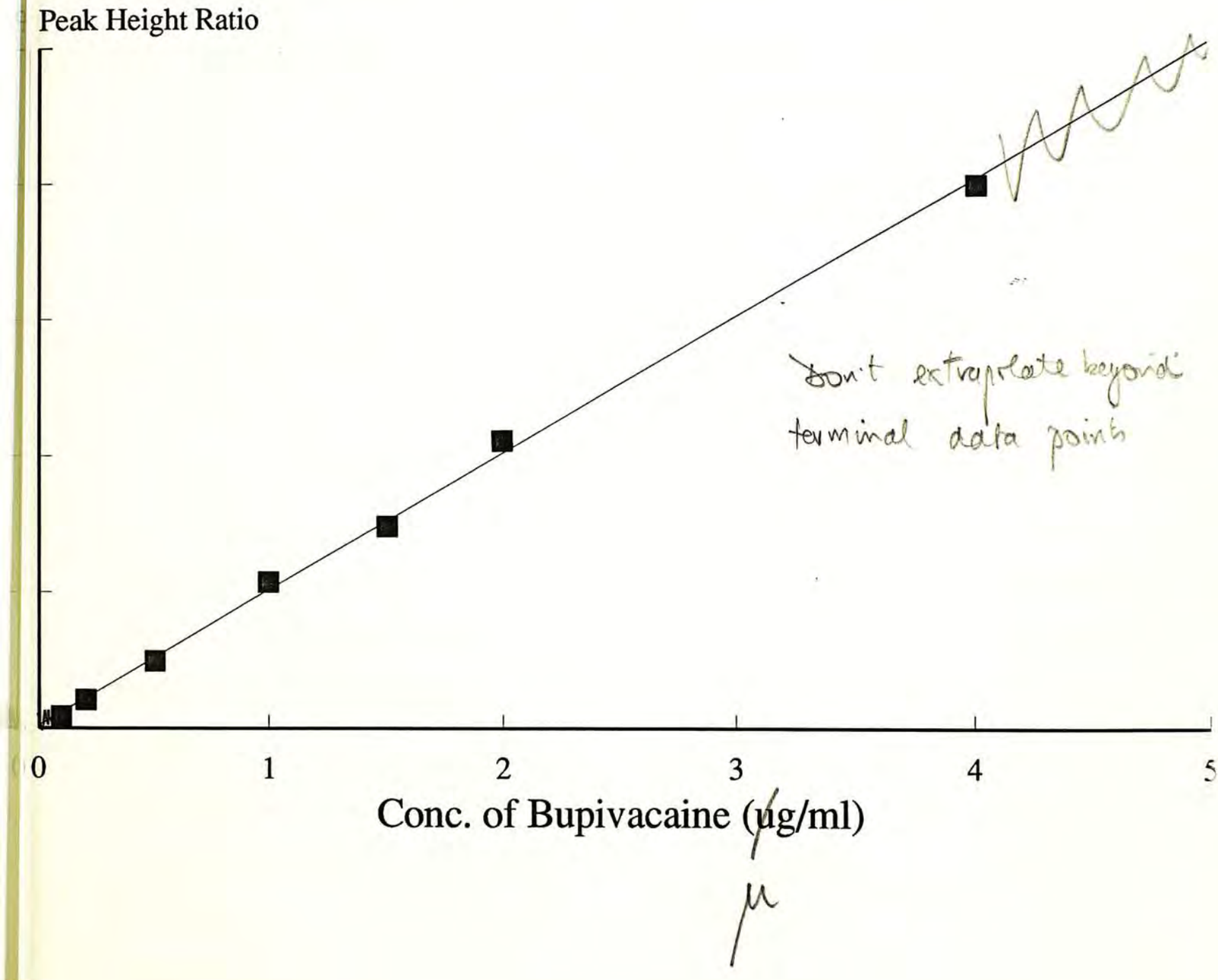
Calibration and precision of the GC assay (n*=6).

Concentration (μg/ml)	Bupivacaine to etidocaine peak-height ratio (mean ± S.D.)	Relative standard deviation (R.S.D.)
0.1	0.095±0.005	5.8
0.2	0.200±0.008	4.1
0.5	0.495±0.022	4.4
1.0	1.070±0.050	4.6
1.5	1.485±0.044	3.0
2.0	2.093±0.054	2.6
4.0	3.972±0.081	2.0
Batch standard# at 2.0 μg/ml	2.090±0.144	6.9
Calibration graph	y=0.9957x+0.0190 (r=0.9994)	

* Number of determinations for each concentration.

\# Duplicate determinations done daily for five consecutive working days to indicate day-to-day variation.

Fig 6. A calibration curve of peak height ratio (bupivacaine/internal standard) against the drug concentration (μg/ml) of bupivacaine in plasma.



time between the groups (Table A1, p.184). Derived pharmacokinetic variables were presented in Table 4. The addition of adrenaline did not alter the Clp, Vss and $t_{1/2}$ of bupivacaine. Peak plasma concentration (Cmax) were significantly lower in the adrenaline group (mean±S.D. [range]: 2.57±0.61 [1.52-3.11] μg/ml) and the time to maximum concentration (tmax) was delayed (median [range]: 25 [15-30] min) compared with the control group (3.22±0.27 [2.84-3.53] μg/ml and 15 [10-20] min respectively for Cmax and tmax) (Fig 7). The difference in Cmax was still significant when adjusted for the initial dose of bupivacaine.

There were no differences in the pain scores between the two groups but the duration of analgesia was variable. Two patients in the control group required morphine 5 mg intraveneously at 30 minutes, although one subsequently required no more opioid analgesia throughout her hospital stay; one patient had excellent analgesia requiring no pethidine, and analgesia lasted 1.5, 7.5 and 12.8 min in the remaining three patients. Duration of analgesia was more consistent in the adrenaline group (range 3.3-10.8 h, median 9.2 h). Two patients in the adrenaline group requested no further pethidine after the first dose.

There were no signs of local anaesthetic toxicity after interpleural injection. Two patients complained of transient retrosternal chest discomfort, but clinical examination and chest radiographs failed to reveal any abnormality. No air was aspirated from the catheters, all of which were removed intact.

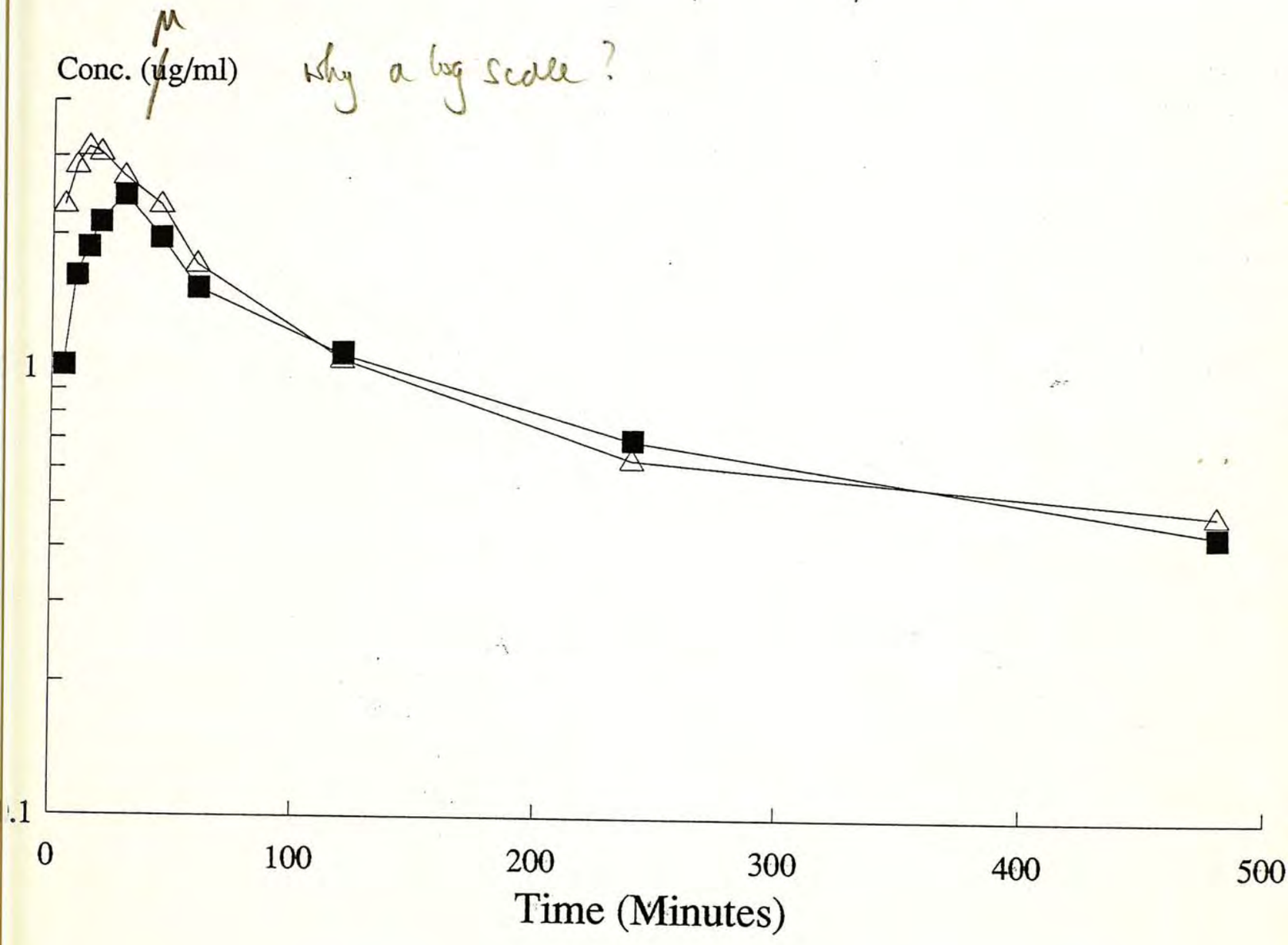
Table 4.

Pharmacokinetic variables in patients after interpleural injection of 0.5% bupivacaine 2.5 mg/kg without (control) and with added adrenaline 1: 200,000. Total body clearance (Cl), apparent volume of distribution at steady state (Vss), elimination half-life ($t_{1/2}$) and peak concentration (Cmax) are presented as mean±S.D. Time to peak concentration (tmax) is given as median (range).

Pharmacokinetic parameters	Control (n=6)	Adrenaline (n=6)	p values
$AUC_{0-\infty}$ (µgmin/ml)	595.3±144.8	592.7±181.9	≥ 0.05
Clp (ml/minkg)	4.60±1.92	4.67±1.83	≥ 0.05
Vss (l/kg)	1.70±0.91	2.05±0.93	≥ 0.05
$t_{1/2}$ (min)	234.8±42.6	278.8±50.4	≥ 0.05
Cmax (µg/ml)	3.22±0.27	2.57±0.61	≤ 0.05*
tmax (min)	15 (10-20)	25 (15-30)	≤ 0.05*

* Indicates significance exists.

Fig 7. **Mean±S.D. venous plasma concentration-time profiles after interpleural administration of bupivacaine 2.5 mg/kg plain (△) and with 1: 200,000 adrenaline (■).**



2.4. DISCUSSION

As mentioned before, packed columns have higher sample capacity and are not easily overloaded. The use of packed columns is relatively cheaper compared with capillary columns and thus they are found to be suitable for economic routine analysis, particularly in those laboratories which are not well equipped. The retention times of the internal standard and bupivacaine were 3.0 and 7.2 min respectively, which meant that the total running time was about 10 min only. Therefore the assay could be achieved in a short period of time (rapid extraction and chromatographic analysis required 20 and 10 min respectively) and can cope with emergency as well as routine drug monitoring or pharmacokinetic study. The relative standard deviation for the calibration standards was lower than 5.8% and the between batch variation (inter-assay variability) was only 6.9%. These results demonstrated the high accuracy and reproducibility of the described method. The minimum detection limit was around 0.01 μg/ml by using 0.5 ml plasma which was lower than the methods reported by Berlin *et al* (25) and Desch *et al* (26) and comparable with those using capillary chromatography (22-23). A detection limit of 0.001 μg/ml using capillary GC has been reported (24), with a coefficient of variation of 15% so that the obtained value was very dubious. Although greater sensitivity might be possible by increasing the volume of plasma used in the analysis, the proposed method was sensitive enough to measure plasma bupivacaine in surgical patients who were treated with this local anaesthetic. The linear calibration range was sufficiently wide (0.1-4.0

μg/ml), further indicating the high versatility of the GC method.

The individual plasma bupivacaine concentrations of each patient of the two groups were presented in Tables A2 and A3 (p.185-186). The addition of adrenaline delayed and decreased peak concentrations of bupivacaine after interpleural injection (Fig 7). Absorption of interpleural bupivacaine appeared to be as rapid as that following intercostal block (46) with tmax usually between 10 and 20 min after both routes. Peak venous plasma concentrations of bupivacaine were high compared with other studies. The potential range for CNS toxicity was estimated to be 2-4 μg/ml (46). Cmax varied widely between studies, partly because of different doses, long intervals in blood sampling and interpatient variability. In thoracic surgery, there is variable damage to lung tissue, dilution of local anaesthetic by blood and loss from the chest drain. Greater concentrations than ours have been reported (37) in adults and peak arterial concentrations of 4-7 μg/ml after interpleural infusion caused no complication (47). There is continued debate on the optimal dosage, volume and concentration of local anaesthetic solution given, although the minimum effective dose should be used. Animal studies demonstrate that the mechanism of interpleural analgesia is multiple intercostal block (48) and an increased volume cause a more extensive block (49). Quality of analgesia after 0.25% and 0.5% bupivacaine may be similar (41).

We calculated the interpleural dose and volume of bupivacaine solution used from the patient's weight. Weight has been shown to affect the

pharmacokinetics of local anaesthetics (46), but it is logical that weight should have some effect on Cmax. If the same dose is given to two patients with markedly different body weight, we would expect the peak concentration to be comparatively lower in the heavier patient if the rate of absorption is similar. The drug would distribute through a greater volume of distribution in the heavier patient before achieving a peak concentration. Patient's weights in this study ranged from 46 to 80 kg and we did not wish to give the same dose to the patients at each extreme. Therefore we have adopted the dose by weight regime.

Pharmacokinetic variables for interpleural bupivacaine with adrenaline have been estimated in Caucasian patients (36). Tmax and Vss were similar, but Clp and $t_{1/2}$ were greater ($p<0.05$) compared with our patients. Cmax was not significantly different ($p=0.12$). The patients were much younger than our patients and no weight data were given. Details of general anaesthetic technique were not provided and all patients were given bupivacaine 150 mg with a greater concentration of adenaline (1:100,000). However, ethnic race, age and weight are not usually significant factors affecting the pharmacokinetics of local anaesthetics (46). We have no other explanation for the differences in Clp and $t_{1/2}$ and a further comparative study would be required to clarify this.

Efficacy of analgesia has been variable and this is apparent even in series from proponents of interpleural analgesia. Effective pain relief in 78 of 81 patients, with a mean duration of 10 hours, was reported initially (50), but subsequent papers by the same group were less consistent. There was

unsatisfactory analgesia in two of the 30 patients, with a further six requiring additional analgesia within 4 hours (41). Total postoperative analgesic requirement was not decreased by interpleural bupivacaine (51), although reduced morphine usage has been shown in the first 4 hours after operation (52). After cholecystectomy, three of 11 patients required additional opioid analgesia (36) and there was no pain relief in five of 24 patients after thoracotomy (43). Inadequate analgesia (40,42,53), pneumothorax and lung trauma (54) have been common problems in other studies.

The addition of adrenaline has been postulated to improve the quality of analgesia: by decreasing the absorption of bupivacaine, it might provide increased time for the local anaesthetic to diffuse through the pleura to the intercostal nerves. This has not been apparent in the present study or in the previous work (43).

In summary we described here an accurate, rapid, simple and sensitive method for the gas chromatographic determination of bupivacaine in human plasma, using n-hexane as the single-step extraction solvent from alkaline medium and an inexpensive packed glass column. We also found that adrenaline seemed to be useful to avoid toxic effects of bupivacaine during the early stage of interpleural analgesia. For those workers and situations where interpleural analgesia with bupivacaine is found to be a useful technique, we recommend the addition of adrenaline 5 μg/ml to delay absorption and decrease peak drug concentrations.

2.5. CONCLUSION

A sensitive and selective gas-liquid chromatographic method for the determination of bupivacaine in human plasma was described. The technique was based on a simple extraction of the drug and the internal standard, etidocaine, from alkalinized plasma with n-hexane. The gas chromatograph was equipped with a glass column (2.0m x 2mm) packed with 3% W/W SP 2250 on Chromosorb W (80/100 mesh), and a nitrogen detector. The method could accurately measured plasma bupivacaine concentrations down to 0.01 μg/ml. The day-to-day variation of bupivacaine at 2.0 μg/ml was 6.90% (n=10). The calibration graph was linear over the range of 0.1 to 4.0 μg/ml and the recovery at 2.0 μg/ml was 91.9±2.8% (n=6). The method was accurate, fast and sensitive, and was suitable for the therapeutic monitoring of patients who were chronically treated with bupivacaine for pain relief and for pharmacokinetic study of the drug.

In this study, bupivacaine, 2.5 mg/kg (0.5 ml/kg of 0.5% solution), with or without adrenaline 5 μg/ml, was administered by interpleural injection to 12 patients after elective cholecystectomy. Kinetic analyses using the non-compartmental technique indicated that addition of adrenaline had no effect on total body clearance, apparent volume of distribution at steady state or elimination half-life of bupivacaine. However, the mean (± S.D.) peak plasma concentration (2.57±0.61 and 3.22±0.27 μg/ml, with and without adrenaline respectively) was significantly lower in the adrenaline group and

the mean (± S.D.) time for maximum absorption (24.7±2.7 and 15.0±1.3 min respectively) was longer when adrenaline was co-administered. Analgesia was variable and no differences were detected between the two groups. The addition of adrenaline appears prudent to minimize possible bupivacaine toxicity.

CHAPTER 3

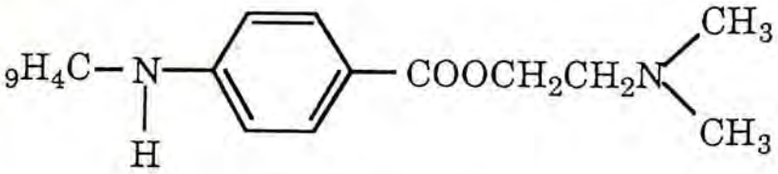
GAS-LIQUID CHROMATOGRAPHIC DETERMINATION AND PHYSICOCHEMICAL STUDIES OF SIX CLINICALLY USED LOCAL ANAESTHETICS

3.1. INTRODUCTION

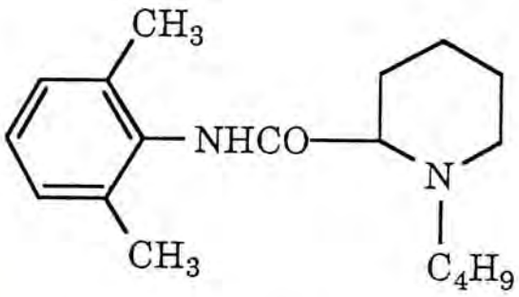
Local anaesthetics are mainly used for regional analgesia during surgery or for post-operative pain relief. As mentioned in Chapter 1, these clinically useful agents essentially fall into two chemical categories: amino esters and amino amides. The chemical difference between the heterologous group is reflected biologically in the rate of metabolism such that ester compounds are hydrolysed in plasma, whereas amide compounds undergo enzymatic degradation in the liver. Alteration in the chemical structure within an homologous groups produces quantitative changes in the pharmacological potency and rate of metabolism and the type of metabolites formed which will affect systemic toxicity. Therefore the judicious use of different local anaesthetics in hospital practice not only requires the technical skill in the performance of the different nerve blocks and a careful evaluation of the patients' clinical statues, but also the thorough understanding of their pharmacological properties (54). On the other hand, it is known that the action of a drug in the body (pharmacological activities) and the characteristics of disposition are determined to a large extent by its physicochemical properties. This valuable knowledge will help to interpret

and possibly predict the behaviour of a drug after administration to the body. Although a lot of work has been done on the clinical evaluation (55) and pharmacokinetics of local anaesthestics (46,56-57), data of their physicochemical properties are not plentiful. In order to bridge this gap, six common local anaesthetic agents, having similar chemical struture (Fig 8) and clinical usage, ~~have been~~ were chosen for the present investigation. They included amethocaine (Am), bupivacaine (Bu), etidocaine (Et), lignocaine (Li), mepivacaine (Me) and prilocaine (Pr). We found that the published chromatographic methods (58-60) for measuring either one of the six local anaesthestics was not suitably applicable in the simultaneous assay. Although methods concerning the simultaneous determination of some local anaesthetics by GC (61-64) and HPLC (65-66) have been reported recently, we are not aware of a chromatographic method for these six local anaesthetics in biological samples in the literature. To facilitate the study, we have developed a simple, reliable, and sensitive gas-liquid chromatographic method for the simultaneous determination of the six local anaesthetics mentioned above, and applied the method to investigate the partition coefficients (K) in various organic/aqueous systems and the buccal absorptions of the drugs. The results so obtained together with the pKa values were employed to find out if there is an inter-relationship among these parameters and a correlation with the pharmacological activities like the duration of action, onset of action and anaesthetic potency.

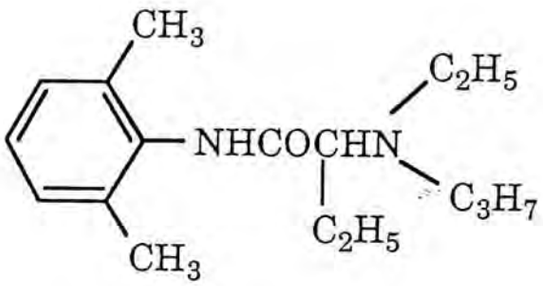
Fig 8. Chemical structures of the six clinically-used local anaesthetics and the internal standard, clomipramine.



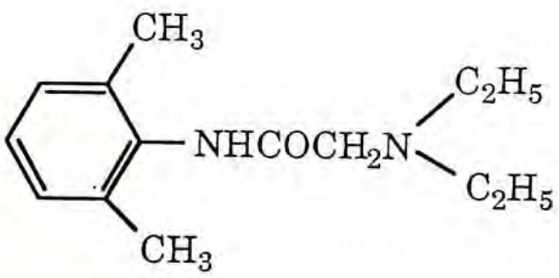
Amethocaine



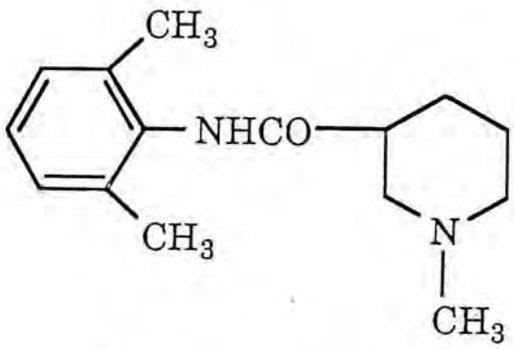
Bupivacaine



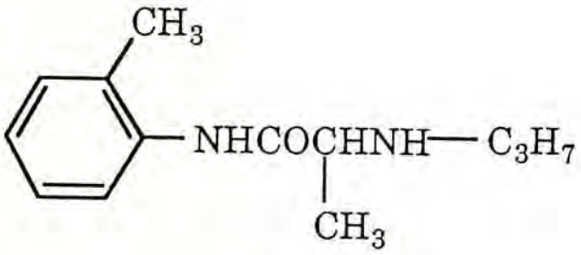
Etidocaine



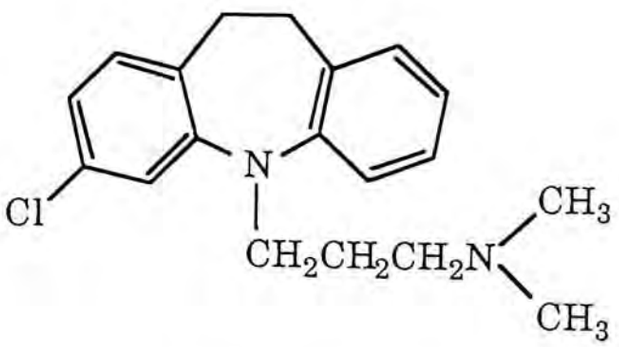
Lignocaine



Mepivacaine



Prilocaine



Clomipramine

3.2 EXPERIMENTAL

3.2.1. Reagents and apparatus

Amethocaine, bupivacaine, clomipramine, etidocaine, lignocaine, mepivacaine and prilocaine hydrochlorides were supplied by Astra Pharmaceutical Production AB (Sweden). AnalaR-grade dichloromethane, chloroform, diethyl ether, n-hexane, methanol, octanol were supplied by E. Merck (Darmstadt, F.R.G.), hydrochloric acid and sodium hydroxide by BDH (England). Disodium citrate, disodium phosphate, double distilled water, glycine and monopotassium phosphate. The following glassware and instruments were used: 15-ml capacity Quick-fit evaporating tubes with tapered base of 50 µl, 15-ml capacity centrifuge tubes with well-fitted screw caps lined with Telfon septa (Pyrex, England), automatic tilt shaker and pH meter. All glassware was cleaned and silanized with 3% HMDS in chloroform before use.

3.2.2. Gas Chromatograph

The gas chromatograph was a Varian Model 6000 (Varian Association, Palo Alto, CA, USA) equipped with a nitrogen-phosphorus detector. The coiled glass column (2m x 2mm i.d.) was packed with 3% W/W SP2250 material on Chromosorb W, 80-100 mesh. The column was silanized with 2 x 50 ml 3% HMDS and conditioned at 320°C overnight before use. The analysis was performed with temperature programming such that the column temperature was initially kept at 210°C for 5 min, then raised to 280°C

for 3 min at the rate of 10°C/min. The injector and detector temperature were maintained at 300°C and the gas flow rates were: nitrogen (carrier gas) at 30 ml/min; air at 175ml/min and hydrogen at 4.5 ml/min. The signal was recorded and displayed by a flat-bed recorder (Linseis, Model L6512).

3.2.3. Calibration and general analytical procedures

A stock solution of drugs containing 1 mg/ml of each of the six chemicals in methanol was prepared. Working drug standards were prepared by spiking 0.5 ml of diluted saliva solution with appropriate microlitres of the stock solution to obtain seven standards with the following concentrations: 0.1, 0.2, 0.5, 1.0, 1.5, 2.0 and 4.0 μg/ml. A quantity (1.5 μl) of 0.4 mg/ml clomipramine HCl as internal standard was added to each standard followed by the addition of 30 μl of 5M sodium hydroxide solution. The alkalined samples were extracted with 5 ml n-hexane with the aid of a mechanical shaker for 10 min and then centrifuged in order to separate the two layers. The organic extract was dried under a gentle stream of nitrogen at 45°C in a water bath. The residue was redissolved in 10 μl of methanol and an 1-μl aliquot was injected onto the GC for analysis.

Recovery of the drugs using this procedure was estimated by comparing values obtained from spiked samples after extraction with those adding in n-hexane solutions without extraction step (100 percent) at the concentration of 2.0 μg/ml. Precision was also checked at the concentration of

2.0 μg/ml before and after the analysis everyday.

3.2.4. The determination of pKa

The pKa values were determined using a simple acid-base titration method according to Cookson (61). Six local anaesthetic hydrochlorides equivalent to 5×10^{-4} mol were dissolved separately in 25 ml aliquots of carbon dioxide-free distilled water in volumetric flasks. Each solution of the drug was titrated at 25±1°C with 9.763×10^{-3} M sodium hydroxide solution (which was initially standardized with 0.0100 M HCl). The base was added in successive 2.5 ml quantities with the pH being recorded after each addition. The pKa was the value corresponding to the pH at 50% neutralization. A blank titration was carried out using 25 ml methanol alone. The above pKa determination for each drug was repeated two times using fresh solution.

3.2.5. The determination of partition coefficients

A mixture of the six local anaesthetics (each containing 0.01 mg/ml) in 5 ml of Sφrensen phosphate buffer at pH 7.4 was placed in a glass centrifuged tube containing 5 ml of dichloromethane (previously saturated with buffer). The tube was tightly screw-capped and shaken for 24 hours, after which the tube was centrifuged to ensure complete separation of the two phases. Tubes containing the mixture of the six drugs in the same buffer were stored at 25°C and -20°C as control samples. The initial (C_I) and final (C_F) concentrations of the drugs in the buffer were determined by taking 0.5-ml

aliquots of the aqueous layers in the control and working samples respectively, and using the procedures described under the Analytical Section 3.2.3. The values of K were then calculated from the equation:

$$K = (C_I - C_F)/C_F \ldots\ldots [15]$$

The whole procedure was repeated by replacing dichloromethane in turn with chloroform, diethyl ether, n-hexane and octanol. Each analysis was performed in a set of triplicate.

3.2.6. The buccal absorption tests (B.A.T.)

These were carried out according to the procedure described by Chan (69). Oral solutions containing the drugs alone or a mixture of the six, were prepared by dissolving the drugs in distilled water. Each solution contained 2.0 mg of drug per ml. An aliquot of the drug solution (0.5 ml) was diluted with 24.5 ml of the appropriate buffer solution (pH 3.0-9.0) in a 25 ml volumetric flask. Sφrensen buffer of pH 3.0 to 5.0 were prepared from disodium citrate and hydrochloric acid (1M); pH 5.0 to 8.0 from disodium and monopotassium phosphates; pH 8.0 to 9.0 from glycine and sodium hydroxide (1M). The resultant solution was then placed in the subject's mouth and circulated about 300 times around the mouth by the movement of cheeks and the tongue during a 6 minutes period, after which the solution was expelled into a clean beaker and the mouth was rinsed with 10 ml of distilled water for 10 seconds. The rinse was expelled into a second beaker. The volume and pH of the expelled solution in the first beaker

were measured immediately. The solutions in both beakers were then combined, adjusted to 250 ml with distilled water and analyzed for drugs using a 1-ml aliquot (following the procedures in the Analytical Section 3.2.3.). During the waiting time between successive tests (30 min), the mouth was rinsed with three changes of distilled water. A blank solution representing the initial concentration of the drugs before B.A.T. was prepared by taking 0.5 ml of the drugs solution (2.0 mg/ml) and adjusting to 250 ml with distilled water and 1-ml aliquot was used for analysis, again following the procedures in the Analytical Section 3.2.3. The absorption of the six drugs in the B.A.T. was taken to be the difference between the amount introduced and the amount remaining in the expelled solution. Two healthy male volunteers who had fasted and cleaned their buccal cavities before the experiment took part in this buccal absorption test.

3.3. RESULTS

3.3.1. Gas chromatograph

Two types of GC columns (3% W/W OV17 and 3% W/W OV101) were initially tested but the six drugs were not successfully resolved. During the course of development of the GC method for the determination of bupivacaine in plasma (Chapter 2), various local anaesthetics were screened as possible internal standard. It was found that lignocaine, prilocaine and mepivacaine could be assayed by the same GC method using a SP2250 column. Thus the simultaneous determination assay of the six local anaesthetics in this study was developed based on the established bupivacaine method, which required n-hexane as the extracting medium.

As shown in Fig 9, the analytical peaks of Pr, Li, Et, Me, Am, Bu and clomipramine were well resolved with good symmetry and good peak shapes. The retention times were 4.0, 4.6, 6.6, 8.7, 10.5, 11.1 and 12.7 min respectively. The calibration graphs (Table 5) covering the concentration range of 0.1-4.0 μg/ml were linear for all six compounds with acceptable coefficients of linear regression between 0.9968 and 0.9999. The between-day coefficients of variation of the six local anaesthetics at 2.0 μg/ml were satisfactory (ranging from 2.2 to 7.1%) which indicated that the assay was reliable and reproducible and their recoveries were reasonably high (ranging from 76 to 95%).

Fig 9. **Gas chromatogram of the six local anaesthetics under the optimized conditions. 1, prilocaine (0.8 μg/ml); 2, lignocaine (0.8 μg/ml); 3, etidocaine (0.7 μg/ml); 4, mepivacaine (0.7 μg/ml); 5, amethocaine (0.6 μg/ml); 6, bupivacaine (0.7 μg/ml) and 7, clomipramine (internal standard, 0.6 μg/ml).**

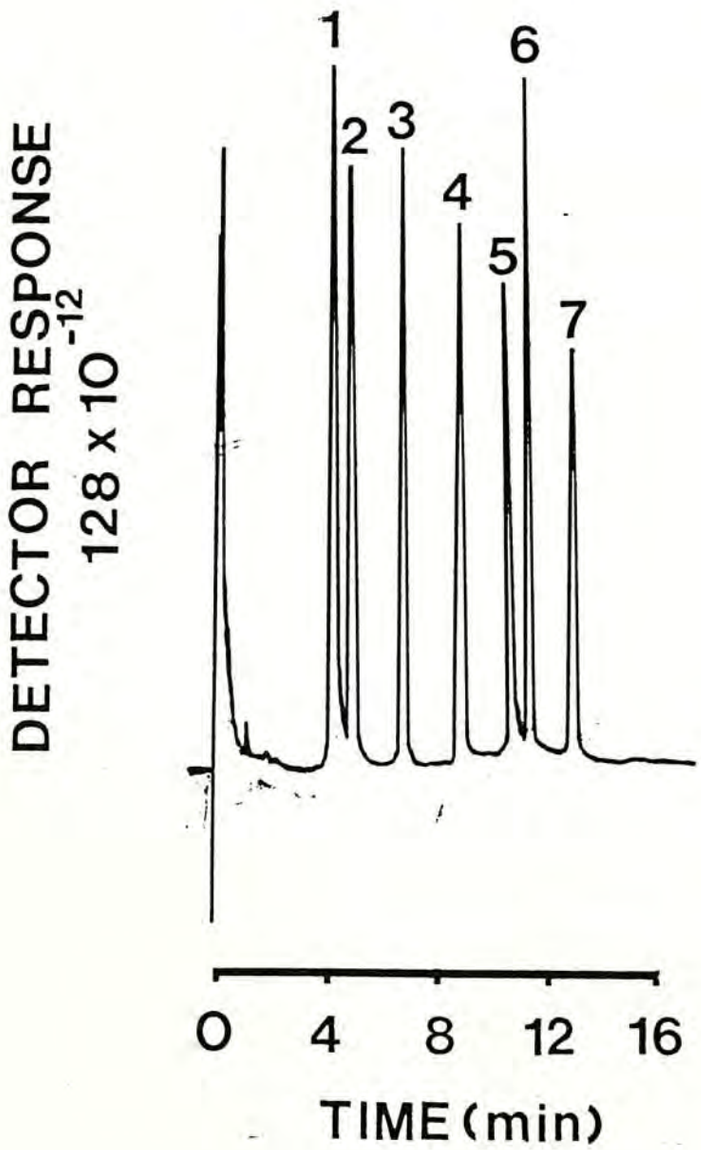


Table 5.

Calibration (mean peak height ratio±S.D.) and precision of the GC assay (n=6).

Conc. (μg/ml)	Am	Bu	Et	Li	Me	Pr
0.1	0.201±0.018	0.250±0.025	0.203±0.013	0.154±0.013	0.174±0.011	0.205±0.015
0.2	0.400±0.018	0.477±0.039	0.415±0.022	0.354±0.010	0.391±0.039	0.443±0.024
0.5	1.032±0.076	1.178±0.086	1.116±0.076	0.881±0.073	0.905±0.027	1.177±0.073
1.0	2.085±0.183	2.300±0.110	2.152±0.145	1.816±0.095	1.816±0.169	2.288±0.079
1.5	2.872±0.171	3.270±0.145	3.078±0.187	2.764±0.095	2.804±0.206	3.260±0.103
2.0	4.178±0.184	4.406±0.194	4.222±0.260	3.648±0.193	3.766±0.130	4.848±0.118
4.0	8.018±0.193	8.554±0.141	8.526±0.339	7.441±0.144	7.572±0.234	8.346±0.147
Batch Std at 2.0 μg/ml	4.206±0.093	4.398±0.096	4.312±0.154	3.677±0.261	3.751±0.224	4.860±0.134
r	0.9995	0.9998	0.9998	0.9999	0.9999	0.9968
Recovery (n=3)	83.1±3.6	94.6±1.4	92.4±2.3	77.5±2.1	80.4±2.0	75.7±2.6

3.3.2. The pKa values

Using the acid-base titration method described, the mean pKa (± S.D.) values (n=3) of amethocaine, bupivacaine, etidocaine, lignocaine, mepivecaine and prilocaine were 8.45±0.24, 8.05±0.13, 7.60±0.18, 7.95±0.09, 7.75±0.11 and 7.90±0.15 respectively, indicating that all drugs are weak bases. In the titrations of amethocaine, bupivacaine and etidocaine hydrochlorides, the free base was precipitated and it was necessary to use methanol to redissolve the precipitates otherwise the pH of the resulting solution was not stable and might affect the accuracy of the results. The addition of methanol did not interfere with the titration results since the blank titration showed that aqueous methanolic solution did not cause deviation to the pH values.

3.3.3. The lipid-buffer partition coefficients and buccal absorption tests

The mean partition coefficients of the six compounds in organic solvent-buffer (pH 7.4) systems varied substanstially (Table 6). However, amethocaine was almost completely tranferred into dichloromethane and octanol with K approaching infinity. Similar observation occurred in bupivacaine, etidocaine and lignocaine with dichloromethane-buffer system. However, the partition coefficients of Am, Bu and Et were generally greater than those of Li, Me and Pr. Hence it could be concluded that the relative lipid solubility of the former group was higher than the latter group. The samples at -20°C served as 100% of the starting materials while samples at 25°C were controls to check if the drug would be decomposed in buffer at room temperature. Analysis of buffer solutions in the control tubes stored at-20°C

Table 6.

Summary of physicochemical data of the six local anaesthetics.

Parameter	Am	Bu	Et	Li	Me	Pr
Onset of Action*	slow	medium	fast	fast	fast	fast
Duration (min)*	180-600	180-600	180-600	90-200	120-240	120- 240
Relative potency*	4	4	3	1	1	1
pKa	8.45	8.05	7.60	7.95	7.75	7.90
% of buccal abs. at pH 7.4	33.3	36.8	43.2	26.6	21.6	23.4
Partition Coefficient (K)						
(i) CH_2Cl_2/buffer	∞	∞	∞	∞	208	74.5
(ii) ether/buffer	363	236	353	59.3	15.4	27.7
(iii)hexane/buffer	0.64	15.4	56.2	2.08	0.47	0.87
(iv) octanol/buffer	∞	356	492	50.0	22.8	19.1

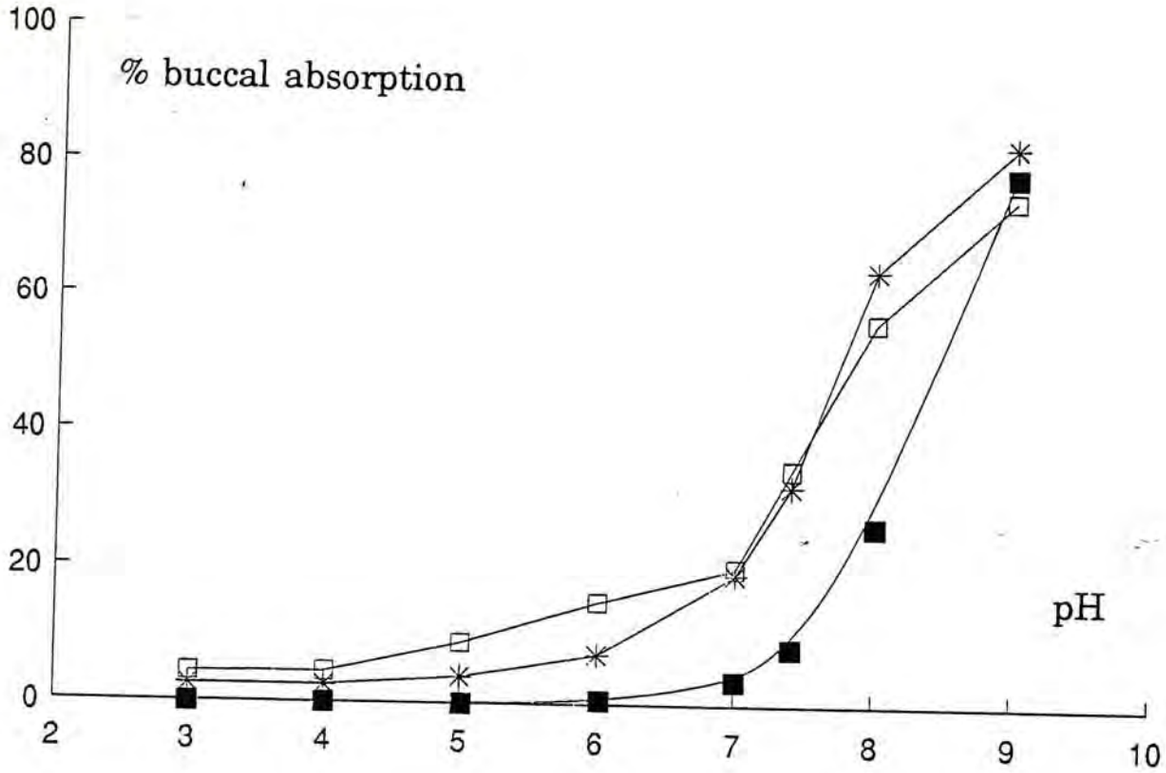
* Information obtained from the literature.

and 25°C indicated negligible decomposition or loss of the drug during the experiment (Table A7, p.190).

The effect of buffer pH on the buccal absorption of the six LA in the two male subjects was shown in Table A8. There was little intersubject variation in the buccal absorption of all these compounds, representing the biological structure of the buccal membrane was almost the same with respect to different individual. The volume of the buffer solution (Table A8) was increased (especially at low pH) during the 6 minutes mouth wash period which was due to the stimulation of saliva secretion, but the change in the pH of the expelled was not very obvious. It could be seen that the percentage of drug absorbed increased as the buffer pH increased. The graphs (Fig 10) of the percentage absorbed of drug against buffer pH take the form of a S-shape with a slow increase initially, followed by a phase of rapid absorption, as shown by the steep slope. The percentage of buccal absorption of the six compounds at pH 7.4 ranged from 21.6 to 43.2%. Whether determined alone or in a mixture of six, the results of buccal absorption were simliar implying no saturation of buccal absorption during successive experiments or the waiting time was long enough for removing the remaining drug inside the buccal cavity.

Fig 10. The effect of pH on the buccal absorption of : (a) amethocaine; (b) bupivacaine; (c) etidacaine; (d) lignocaine; (e) mepivacaine and (f) prilocaine in volunteers I (□) , II (■) and theoretical case (∗).

(a)



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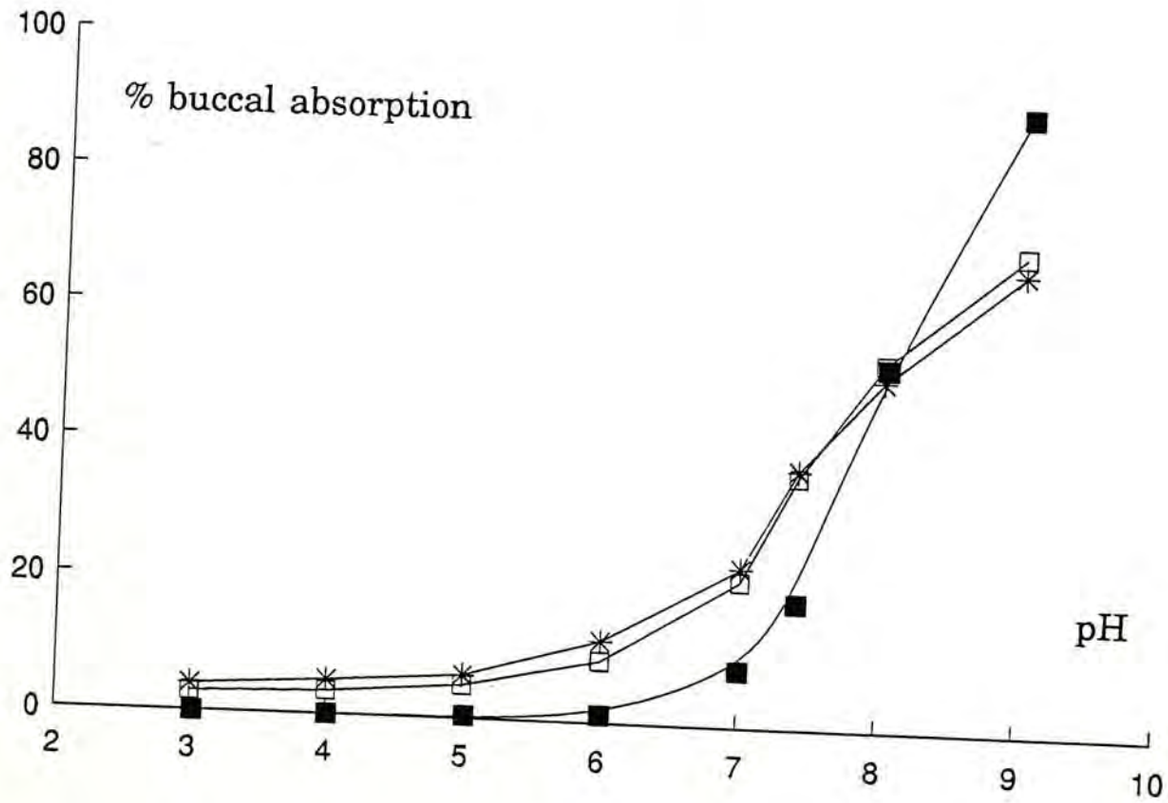
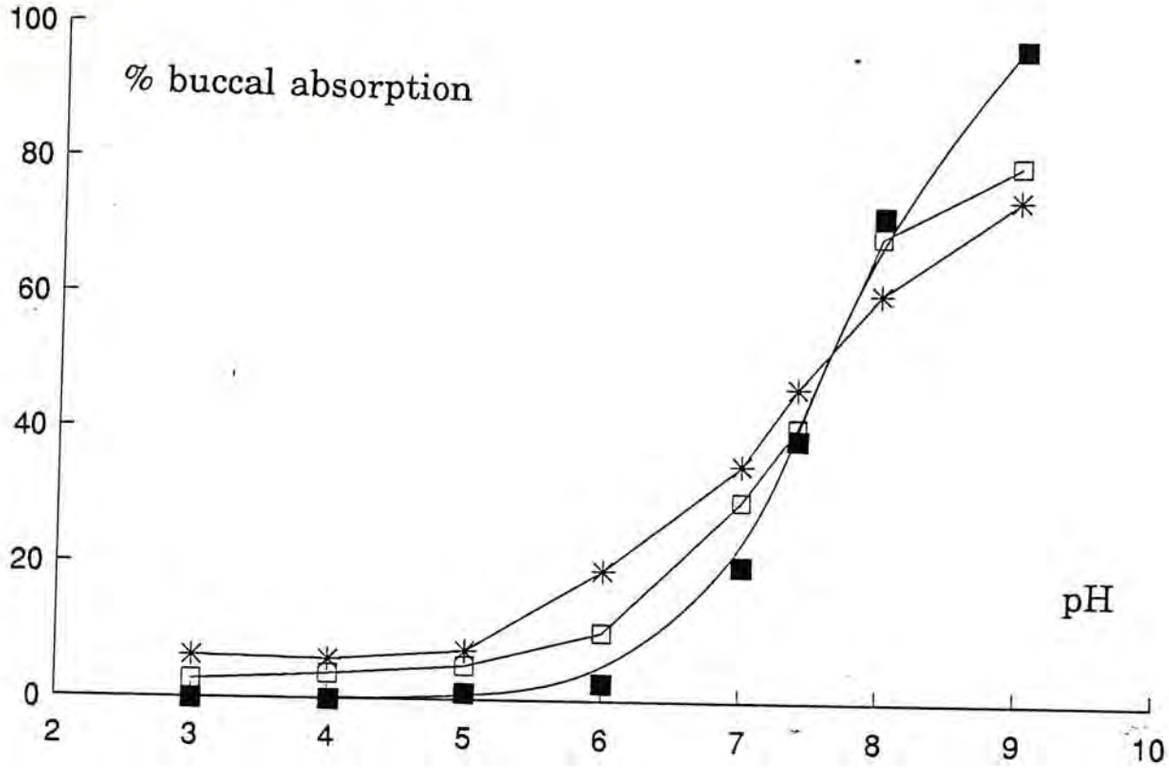
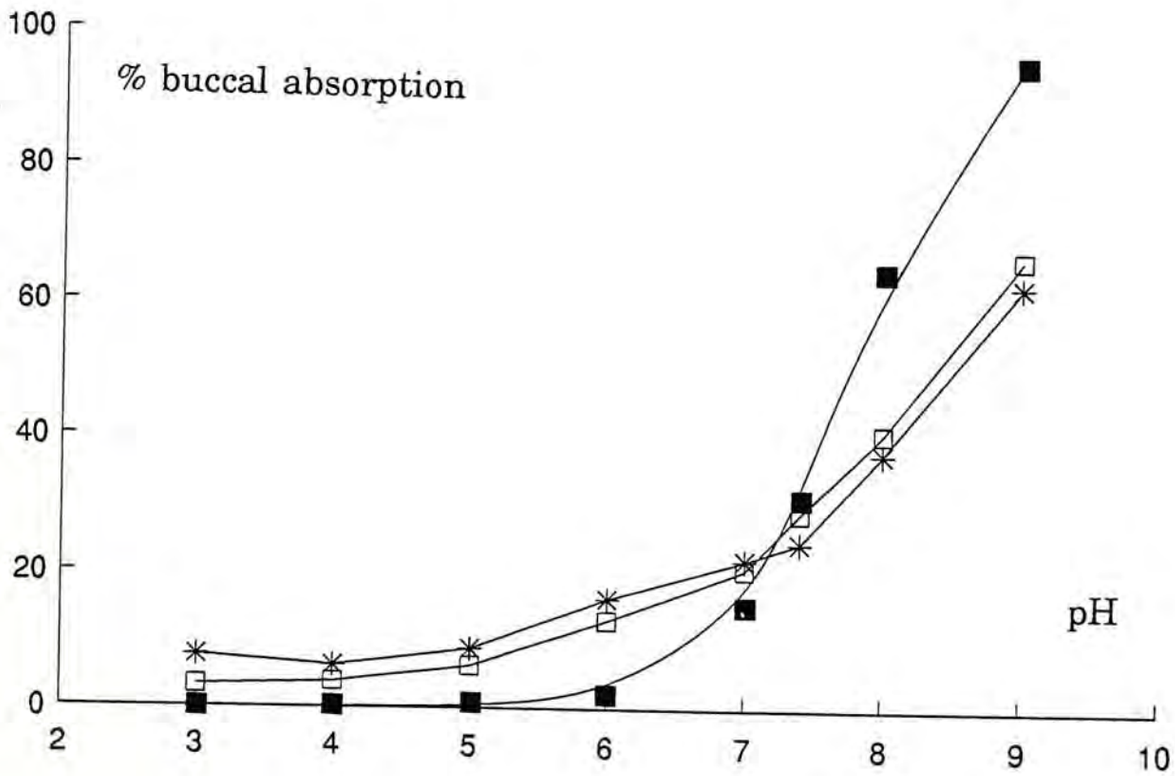


(c)


100
% buccal absorption
80
60
40
20
0
pH
2
3
4
5
6
7
8
9
10

(d)


100
% buccal absorption
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pH
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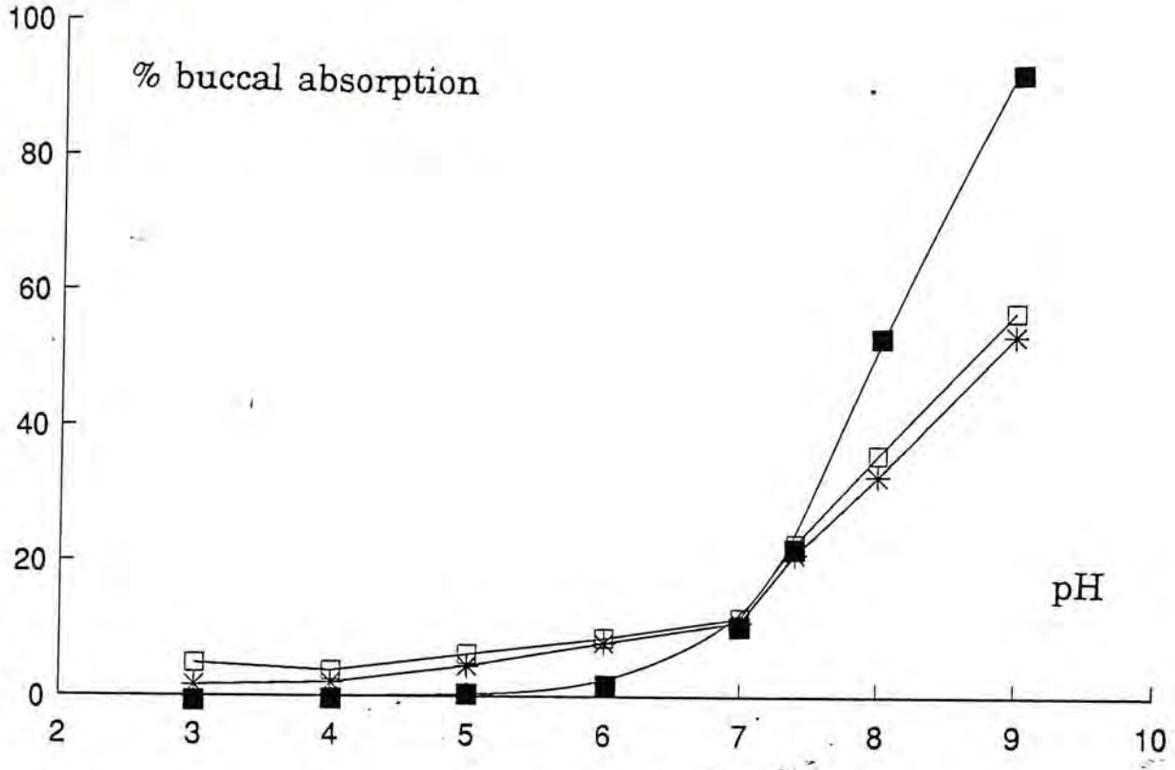
(e)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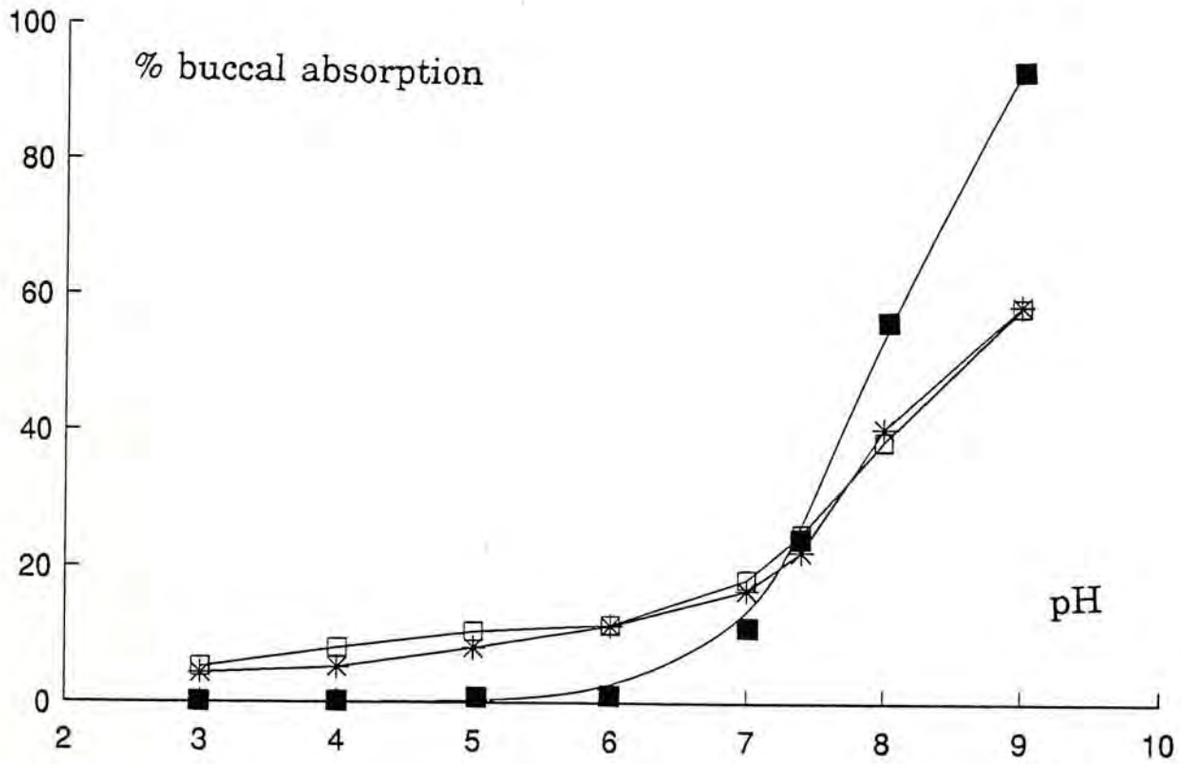
% buccal absorption
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3.4. DISCUSSION

Although we did not perform a preliminary test for optimum mouth contact time for the buccal absorption of the local anaesthetics, we recognized that the contact time can not exceed 10 minutes. For a longer contact time, the volume of saliva secreted into the buffer solution would be increased so that it might overload the capacity of the buccal cavity and disturbed the investigation. The previously reported optimum time for pethidine (69), phenoperidine (70) and ethmozine (71) were around 6 minutes and therefore we expected that this duration would also be suitable for the six local anaesthetics drugs under study. Since more sensitive and better quality pH meter was not available in our laboratory, titration with concentration less than $5x10^{-4}$ mol of the drugs could not be carried out. The procedure for the determination of pKa values in our study was not ideal (68) but it showed similar results when compared with the literature values.

The present study has provided some information on the physicochemical properties of the six LA. A knowledge of these parameters was essential for interpreting the absorption and disposition of the drugs in the body. As mentioned in Chapter 1, the absorption of drugs from the site of application into the blood stream and its distribution, excretion and renal tubular reabsorption are largely achieved by passive diffusion of nonionized molecules across the biological cell membranes. Although some workers (72) have used an animal model with the aid of buccal application cells to investigate this passive diffusion, we believed that B.A.T. was the best *in vivo* model to illustrate passive drug transfer through these biological membranes.

The S-shape of the buccal absorption-pH curves for the six local anaesthetics suggested that the drugs belong to Class Four of Beckett's grouping (73) The percentages of the drug absorbed in this category were a function of buffer pH. The pKa values of the six local anaesthetics showed that they were weak bases which were almost in the ionized form in acidic condition and therefore the rate of diffussion through the buccal membrane was slow. The opposite was observed when the drug was in alkaline medium; the percentage of the nonionized form increased and hence the rate of absorption increased until it reached a saturated level. The behaviour of the ionization of a base was governed by the Henderson-Hasselbalch Equation (Eq. 8, Chapter 1).

The theoretical percentages of absorption of the six LA at different buffer pH were calculated by assuming that only the lipid soluble and nonionized form penetrated the buccal mucosa and the nonionized form was absorbed completely. Using the following equation,

$$\text{Theoretical \% absorption} = \frac{C_1}{C_1 + C_2} \times 100 \quad \ldots\ldots [16]$$

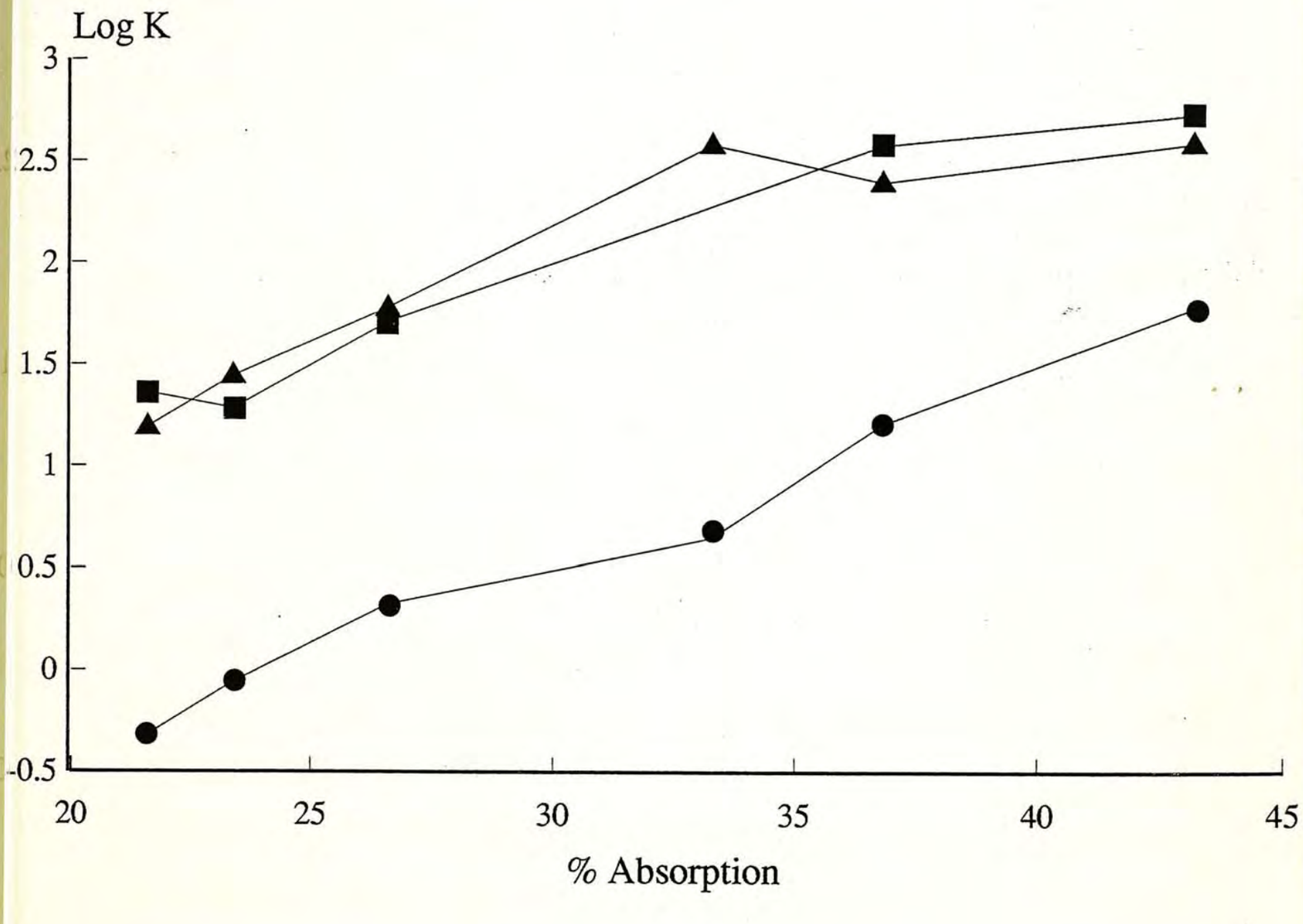
where C_1 and C_2 were the concentrations of drug in the blood and the buccal cavity (buffer solution) respectively. When the theoretical % absorption plotted against buffer pH was compared with the experimental results (Fig 10), it was found that the experimental absorption curves followed the same pattern and shape as the theoretical one, except that the acutal % absorption on the experimental curves were lower at pH 7.4. The discrepancy between the two

curves might be due to the relatively low permeability of the nonionized form across the buccal membranes, which was not accounted for in the equation used for calculating the theoretical % absorption. Another consideration might be that there was an equilibrium of the nonionized form across the buccal membrane. Comparing the % ionized (Table A7) and the B.A.T. graphs above pH 7.0, it was found that although a great proportion of the drugs were in non ionized form, not all of them were absorbed. This might be due to the partition characteristics of the drug between the lipid and aqueous phase, hence the buccal absorption was limited by lipophilic character of the compound.

The results of partition coefficients study showed that among the four organic solvents, n-hexane and Sϕrensen buffer was the best system to illustrate the complicated structure of biological membrane. A relatively good correlation (r=0.991) was observed (Fig 11) between the percentage of buccal absorption at pH 7.4 and the logarithms of K in n-hexane-buffer system. Hence this system could be used as an *in vitro* indicator for the ability of the six local anaesthetics to penetrate the *in vivo* biological barriers.

From the present results and information obtained from the literature (54,74) (Table 6), inter-relationship could be found between the onset of action and the pKa values. Agents with lower pKa values such as Et, Li, Me and Pr tend to possess a more rapid onset of action than agents such as Am and Bu. The reason was that the ionization of lower pKa compounds was greater than that of higher pKa compounds at physiological pH. For instance,

Fig 11. A plot of the mean percentage buccal absorption of the six local anaesthetics at pH 7.4 against their log K values in (▲) ether, (●) n-hexane and (■) octanol-Sϕrensen buffer systems.



at a pH of 7.4, approximately 76% of prilocaine exist in the cationic form whereas over 90% of amethocaine is present in the cationic form. The duration of pharmacological effect was shown dependent on the partition coefficient of the drug molecule; the higher the K values, the longer the duration of action. But it was believed that drug with relatively high K value would penetrate in and out of the membrane readily which resulted in a shorter duration and therefore the result obtained was contrary to the expected one. This could be explained by the fact that the duration of anaesthetic activity was relied on the degree of binding to the membrane and plasma proteins. Addition of larger alkyl substituents to the amine or aromatic end of a compound will normally increase the protein binding ability. For example, the protein binding of bupivacaine and etidocaine exceeds 90%, whereas their homologues, i.e. mepivacaine and lignocaine are only 65 and 75% protein bound respectively. In terms of anaesthetic duration, Bu and Et are 2 to 3 times longer acting than Me and Li. More correctly, it could be said that the duration of the local anaesthetics was not corresponding to its K value but to its protein binding affinity. The result also showed that changes in the lipid solubility of a local anaesthetic would alter its intrinsic anaesthetic potency. As seen in the amide series, the addition of a butyl group to the amine end of Me lead to the formation of Bu, which is much more lipid soluble and potent than Me. It was generally accepted that the excretion of a variety of basic drugs was dependent on the pH of the urine and their physicochemical properties (75). This could be explained in terms of increasing reabsorption of the unionized lipid soluble form of the drug as the

pH of the urine becomes more alkaline (76-77). The normal pH of urine was around 6.5 and therefore a considerable amount of the local anaesthetics would be reabsorbed from the glomerular filtrate according to the data obtained from the B.A.T. If the urine was made acidic, the percentages of the six local anaesthetics eliminated unchanged would increase since less of the nonionized fraction would be available for reabsorption. Conversely, at alkaline urinary pH, reabsorption via the renal tubules would increase and less drug would be eliminated unchanged in the urine. Therefore adjustment of urinary pH to acidic conditions may be used as a method of removing local anaesthetics from the body in cases of acute poisoning.

It can be concluded that the physicochemical properties of local anaesthetics play a very important part in determing their absorption and elimination in the body. It is therefore necessary to consider these factors when one considers the clinical pharmacokinetics of local anaesthetics.

3.5. CONCLUSION

A gas-liquid chromatographic method was developed for the simultaneous assay of six local anaesthetics, including amethocaine, bupivacaine, etidocaine, lignocaine, mepivacaine and prilocaine in biological samples. These drugs and internal standard (clomipramine) in basified samples were extracted into 5 ml n-hexane and the extract was analysed by a temperature programming method (the column temperature was kept at 210°C for 5 min, then raised to 280°C at the rate of 10°C/min) using a 3% W/W SP2250 glass column (2m x 2mm i.d.) connected to a nitrogen sensitive detector. The injector and detector temperature were maintained at 300°C. The pKa values, partition coefficients (K) and the buccal absorptions of the six compounds were also determined in the study. The results showed that the onset of action and the duration could be shown dependent on the pKa values and partition coefficients respectively. A relatively good correlation (r=0.991) was observed between the percentage of buccal absorption at pH 7.4 and the logarithms of K in n-hexane-Sφrensen buffer system and hence the more lipid soluble the local anaesthetic, the greater the buccal absorption. The buccal absorption test supplemented by the n-hexane-buffer partition coefficient could be used as indicator for the ability of the anaesthetic molecule to penetrate biological barriers. The lipid penetration of the drugs and thus their pharmacological action is also influenced by the pKa values of the compound.

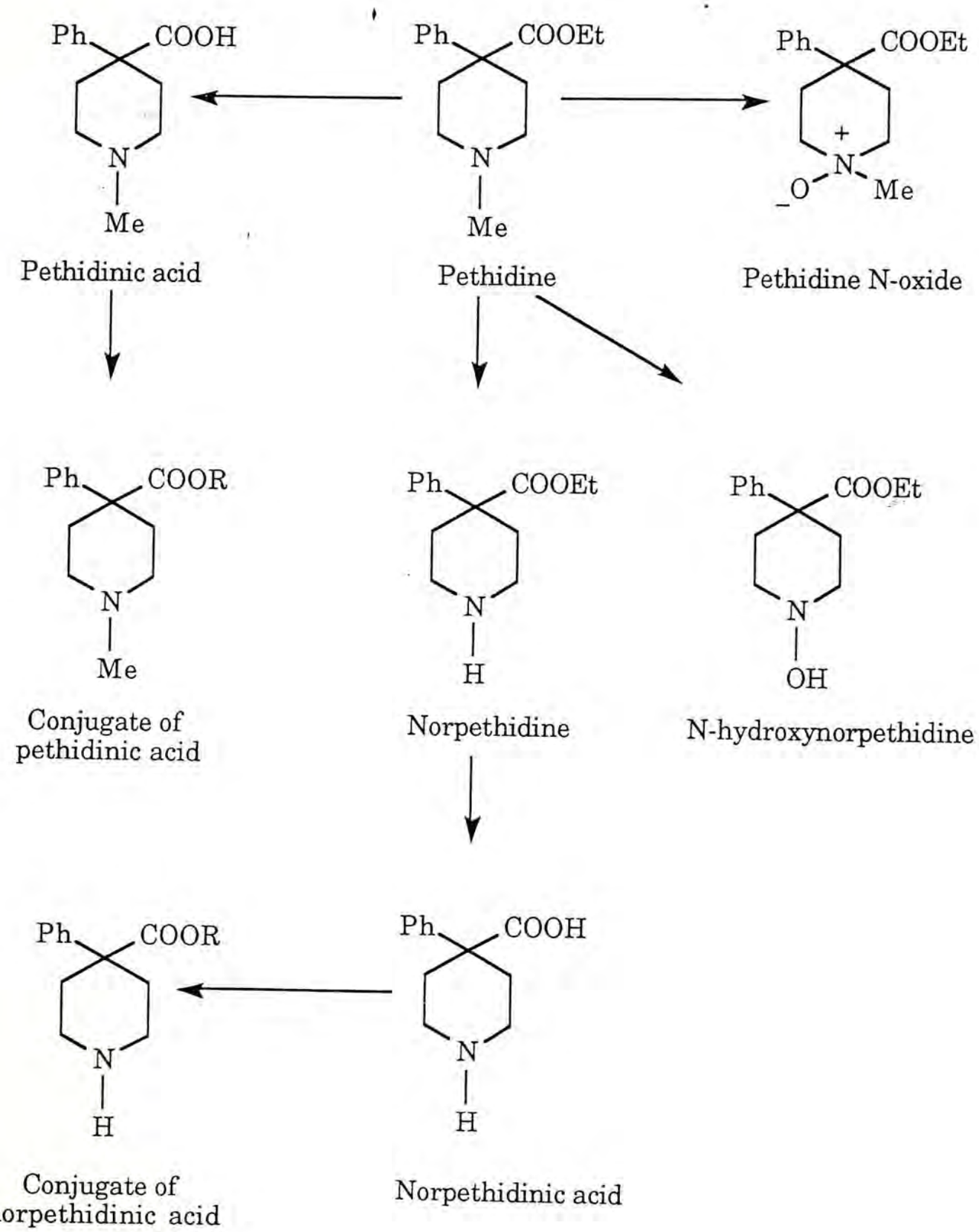
CHAPTER 4

IMPROVED GAS-LIQUID CHROMATOGRAPHIC METHOD FOR THE QUANTITATION OF PLASMA PETHIDINE AND NORPETHIDINE

4.1. INTRODUCTION

Pethidine (PTH or meperidine), was first introduced as a potent analgesic in 1939 by Eisleb and Schaumann (78). It achieves wide therapeutic use in hospital practice (78-79) particularly in the control of post-operative pain and in the relief of many types of moderate to severe pains. Today, over fifty years after its introduction, pethidine is still one of the most commonly used narcotic anagelsic drugs. When pethidine is administered into the body, it is extensively metabolized in the liver, primarily by N-demethylation and hydrolysis. The drug and its metabolites are largely excreted in the urine. The amount of the dose excreted unchanged varies with urine pH, from 0.6 to 27% and the recovery of metabolites is also influenced by the urinary pH (80). Major metabolites include norpethidine (NPTH), pethidinic acid and its conjugate (glucouronide), norpethidinic acid and its conjugate. Other minor metabolites such as pethidine N-oxide (81) and N-hydroxynorpethidine (82) were also isolated (Fig 12). Although most of the metabolites are considered to be inactive, norpethidine, the only metabolite that can be found in the plasma, is estimated to possess half the analgesic potency of pethidine and is twice as potent as a convulsive compared to the parent drug. Hence, measurement of the drug and its N-demethylated metabolite, norpethidine in the plasma of

Fig 12. Biotransformation of pethidine.



patients is of importance in understanding the disposition of the drug/metabolite in the body, and this would allow the development of strategies for avoiding the adverse effects associated with the clinical use of pethidine.

Different types of analytical methods have been reported to determine pethidine in biofluids. Classical spectrophotometic methods such as colorimetry (83-85) and flourometry (86) were considered to be insensitive and non-selective. Carbon-14 radiolabelling technique which was expensive, was claimed to have limited use in man (87). Ion-selective electrodes (88) was reported in pharmaceutical preparations but was not applicable in biological samples. Recently, chromatographic methods have been applied successfully in the determination of nanogram amounts of PTH and NPTH in plasma. These methods involve high performance liquid chromatography with UV detection (89-90), and GC coupled with mass spectrometer (91-95), flame ionization detector (FID) (96-99), electron capture detector (ECD) (100-101) and nitrogen-phosphorus detector (NDP) (102). The polarity of NPTH is relatively high and therefore the chromatographic property of this compound is poor. In the GC methods described above heptafluorobutyric anhydride (98,102), trifluoroacetic anhydride (99), and trichloroethyl chloroformate (100-101) have been employed as the derivatization agents in order to enhance the detectability of NPTH. These procedures were time-consuming and not easy to perform. The other GC method described by Chan *et al* (103), which did not include a derivatization step, was simple and satisfactory, but it required large sample size (5 ml) and four etheral extraction steps for complete

drug/metabolite recovery. We described here a simple, sensitive and accurate GC method for the measurement of plasma concentrations of PTH and NPTH simultaneously in surgical patients who have been given a single intramuscular dose of pethidine.

4.2. EXPERIMENTAL

4.2.1 Materials and apparutus

Pethidine hydrochloride was obtained from May & Baker Ltd. (Dagenham, U.K.) , norpethidine hydrochloride from Sterling Winthrop Research Institute (New York, USA) and chlorpheniramine hydrochloride from Astra Pharmaceutical Production AB (Sweden). Glass-distilled AR grade n-hexane and methanol were purchased from Merck (Darmstadt, FRG). The following glassware was used: 15-ml capacity centrifuge tubes with well fitted screw caps containing PTEE linings, 15-ml capacity Quick-fit glass tubes with tapered base of 50 µl. All glassware was cleaned and silanized with 3% HMDS in chloroform before use. The gas chromatograph was a Varian Model 6000 equipped with a nitrogen-phosphoros detector. A coiled glass column (2m x 2mm i.d., 6mm o.d.) was packed with 3% W/W SP2250 on Chromosorb W, 80-100 mesh. The column was silanized with 2 x 50 µl 3% HMDS and conditioned at 300°C overnight before use. The column temperature was maintained at 230°C and that of the detector and injector was at 260°C. The signal was recorded and displayed by a flat-bed recorder (Linseis, Model L6512) The gas flow rates were: nitrogen (carrier gas) at 30 ml/min, air at 175 ml/min and hydrogen at 4.5 ml/min.

4.2.2. General assay procedure

Plasma samples (1 ml) were pipetted into a 15-ml centrifuge tubes with screw cap. Six microlitres of a methanolic solution of 0.025 mg/ml

chlorpheniramine hydrochloride were added as the internal standard, followed by 50 μl of 2M sodium hydroxide solution. The alkaline plasma samples were extracted with 5 ml n-hexane using a mechanical shaker for 15 minutes. After centrifugation, the organic layers were transferred into evaporation tubes and evaporated to dryness in a water bath under a gentle stream of nitrogen at 45°C. The residues were redissolved in 10 μl of methanol, and 2 μl aliquots were injected into the gas chromatograph. The concentrations of PTH and NPTH were determined by using, respectively, drug/marker, or metabolite/marker peak height ratios from calibration graphs.

4.2.3. Pharmacokinetics of pethidine in patients

In this study, with the approval of the local Research Ethical Committee, five Chinese patients (sex, 4M/1F; age, 38.4±8.4 yrs; weight, 59.9±11.3 kg) who underwent either hernia repair or excision exostosis, and subsequently received single dose of pethidine (1 mg/kg) intramuscularly. The patients were initially anaesthetized by 200 mg of thiopentone and a mixture of dinitrogen oxide, oxygen and halothane. The blood samples (5ml) were taken at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12 and 24 hours after dose, and plasma was separated as soon as possible and stored in the freezer at -20°C before analysis.

Pharmacokinetic parameters were obtained from plasma concentration-time points according to standard formulae (10). The areas under plasma concentrations plotted against time (AUC) were calculated

using the trapezoidal rule. The plasma clearance (Clp) was determined according to Eq. 2 (Chapter 1), where Dose was the intramuscularly administered pethidine dose and AUC was the area under the plasma concentration-time curve from zero to infinitive.

$$Clp = \frac{Dose}{AUC} \quad \ldots\ldots [2]$$

The first order rate constants for the decline of plasma concentration after administration were obtained by linear least square regression on log(plasma concentration) against time. The steady state volume of distribution (Vss) was calculated according to Eq. 5 (Chapter 1).

$$Vss = Clp \times MRT \quad \ldots\ldots [5]$$

All these data were computed using the programme PKCALC (45).

4.2.4 Calibration, recovery and precision

Pethidine and norpethidine were dissolved in methanol to make 0.1, 0.01 and 0.001 mg/ml stock solutions. These solutions were subsequently used to spike drug-free plasma in appropriate volumes to obtain six standards with the following concentrations of PTH and NPTH: 10, 20, 50, 100, 250 and 500 ng/ml. A methanolic solution (6 µl) of 0.025 mg/ml chlorpheniramine hydrochloride was added to each sample as internal standard.

Recovery of PTH and NPTH from plasma using this procedure was estimated by comparing values obtained from spike plasma with standard

n-hexane solutions (100 percent). Precision was determined by spiking two replicate samples of PTH and NPTH in blank plasma at the concentration of 100 ng/ml before and after the analysis of patients' samples everyday. The results obtained by the present assay were parallel checked and compared with a modified reference method (103).

4.3. RESULTS

4.3.1 Choice of extraction solvent

Although recoveries were satisfactory (>75%) when using dichloromethane, diethylether and their 3:1 v/v mixture (Table 7), it was found that peaks due to some extractable endogeneous plasma components appeared at about 3 and 15 minutes in the chromatogram. A further purification step such as back extraction was required in order to obtain a better chromatogram. Otherwise these interfering peaks could mask the analytical peak of NPTH as well as lengthen the running time of the whole procedure. We also observed that these solvents produced a strong solvent front signal, resulting in unsteady baseline which could affect the peak height measurements and reduce the accuracy of the results. Other common solvents such as cyclohexane, ethylacetate and n-heptane were suggested not to be good enough for extracting the drugs (104) in plasma sample. Based on the supreb performance of n-hexane in the bupivacaine study, it was chosen as the extracting medium in this experiment even though the recovery of NPTH was slightly lower (NPTH=65%). We avoided using chloroform because this solvent, would react with NPTH chemically to form an extraction artefact, norpethidine ethylcarbamate according to the study of Siek *et al* (105). A greater than 90% conversion of NPTH to norpethidine ethylcarbamate was reported in his study.

4.3.2 Choice of GC system

The polarity of NPTH is relatively high (a secondary amine) and

Table 7.

Comparison of recovery of various organic solvents on PTH and NPTH.

Solvent	% Recovery (Mean ± S.D.) n=5 PTH	NPTH	Comments
Dichloromethane	82.3±3.6	74.8±2.2	The analytical peaks were masked by endogenous substances
Diethyl ether	92.6±2.5	88.6±4.1	Same as above
Diethylether/Dichloromethane (v/v=3:1)	78.6±1.9	80.4±3.3	Same as above
n-Hexane	85.8±2.8	64.9±3.4	No interfering peak was detected

therefore it shows a very poor resolution in most of the GC columns. As discussed before, almost all the GC determinations of NPTH required derivatization. The primary purpose of this sample treatment was to increase the sensitivity of the determination and improve the peak shape in the chromatogram by converting the polar amine group to less polar amide group or its halo-substituted derivatives. The choice of column was very important because a suitable column not only reduces the peak tailing effect of NPTH, but also eliminates the tiresome derivatization treatment. Based on the like

dissolves like principle, we expected that a less polar GC column might elute the NPTH peak well. In order to search for this type of column, we have tested a number of GC systems. As shown in Table 8, the 2% W/W and 10% W/W Carbowax columns were not suitable for the analysis since NPTH appeared to be adhered to the column materials and was not detected. The 3% W/W OV 101 and 3% W/W SE 30 columns gave both tailing and broad PTH and NPTH peaks whereas the 3% W/W OV 17 and 3% W/W OV 25 produced good and symmetrical PTH peak but poor NPTH peak. It implied that these systems were also not adequate for good resolution without the derivatization procedure. It was found that both the 8% W/W Carbowax 20M+2%KOH and 3% W/W SP2250 columns could give good and sharp peaks of PTH and NPTH. In fact, the former has been described (103) to determine PTH, NPTH and pethidine-oxide in biological fluids previously using benzphetamine as the internal standard. As the retention times for both PTH and NPTH are shorter using the 3% W/W SP 2250 column, it was chosen for the present assay. We have also accessed the capability of the two suitable analytical columns and showed that there was one advantage of the SP 2250 over the Carbowax 20M column. The former was stable up to a maximum temperature of 350°C whereas the latter could only tolerate up to 230°C. The operating temperature of the reference method using Carbowax for analysis was around 200°C which rendered decomposition of the stationary phase as we observed that the Carbowax materials must be partly or wholly replaced after using continuously for about two weeks time. On the other hand, the performance of the SP 2250 column was still perfectly applicable for several months, implying

Table 8.

Comparison of the performance of various types of GC systems for the determination of PTH and NPTH.

System	Temp. (°C)	Retention Time (min)		Comments on peak shape
		PTH	NPTH	
1. 2% Carbowax	200	4.4	---	Good PTH, NPTH not resolved
2. 10% Carbowax	200	11.0	---	Same as above
3. 3% OV 17	200	5.6	7.45	Good PTH, broad and tailing NPTH
4. 3% OV 25	170	4.3	6.3	Same as above
5. 3% OV 101	160	3.4	3.8	Tailing NPTH, poor resolution
6. 3% SE 30	180	2.4	4.7	Both tailing and broad
7. 8% Carbowax 20M+2%KOH	200	3.8	7.0	Good PTH and NPTH
8. 3% SP2250	230	3.3	4.5	Same as above

--- Not detected

that the proposed GC system was more suitable for routine pharmacokinetic study of PTH and NPTH. The PTH and benzphetamine peaks were very close and could not be resolved by the SP 2250 column and chlorpheniramine hydrochloride was eventually selected as a suitable internal standard after a series of compounds were tried.

Figure 13 showed the typical chromatograms of extracts from blank plasma and patient's plasma using the above GC system under the optimized conditions. The analytical peaks of PTH and NPTH were well resolved with retention time of 3.3 and 4.5 minutes, respectively. The performance of the GC system used was considered adequate for the assay of PTH and NPTH (Table 9). A plot of peak height ratio of PTH and NPTH to chlorpheniramine was linear over the range of concentration of 10 to 500 ng/ml with % C.V. less than 6.5% in ten determinations. The correlation coefficients of the calibration curves of PTH and NPTH were 0.9992 and 0.9998 respectively. The lowest detection limit was appromixately 5 ng/ml for PTH and 10 ng/ml for NPTH. The results obtained with the present method and the modified reference methods (103) (Figures 14a and 14b) were in close agreement ($r > 0.99$ for both drugs), which indicated that our new GC method was reliable and accurate.

It appears that samples were analyzed by Chan's method also. This is not mentioned in the experimental

Fig. 13. Typical chromatogram of (1) pethidine, (2) norpethidine and (3) chlorpeniramine, the internal standard, after GC analysis of human plasma extracts.

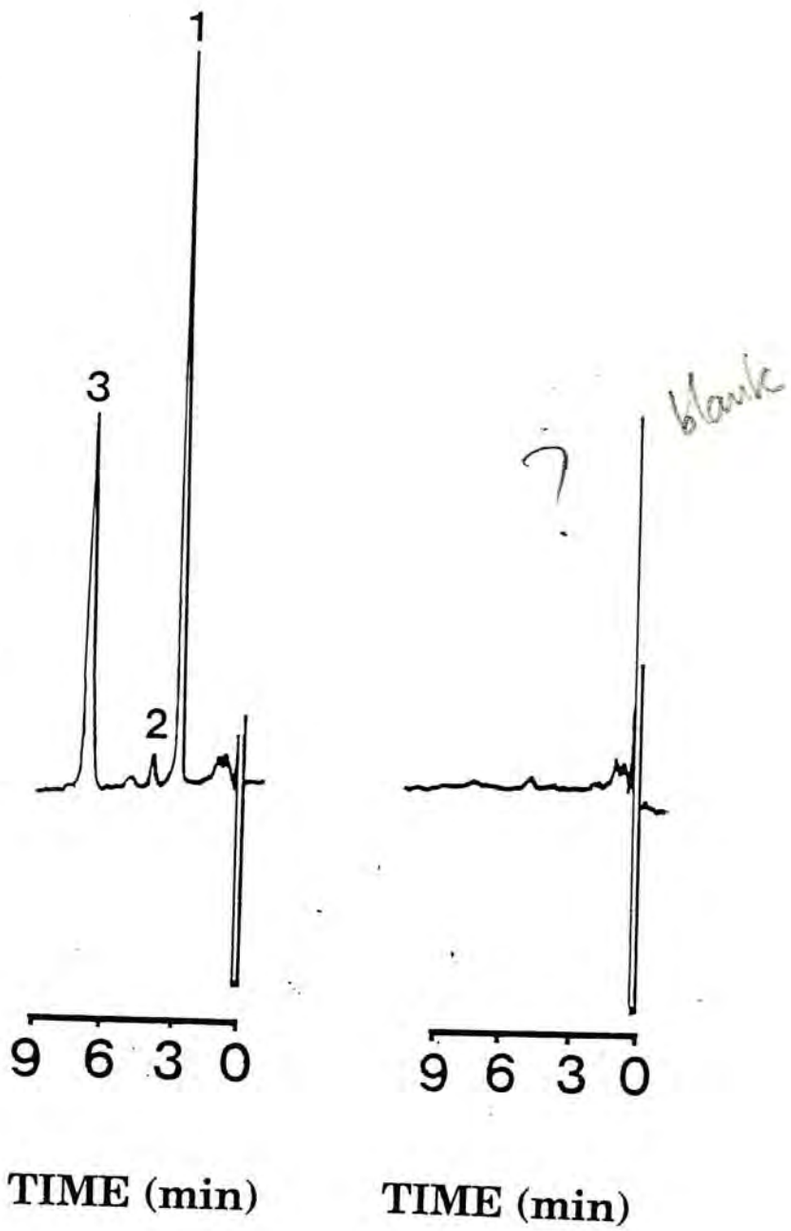


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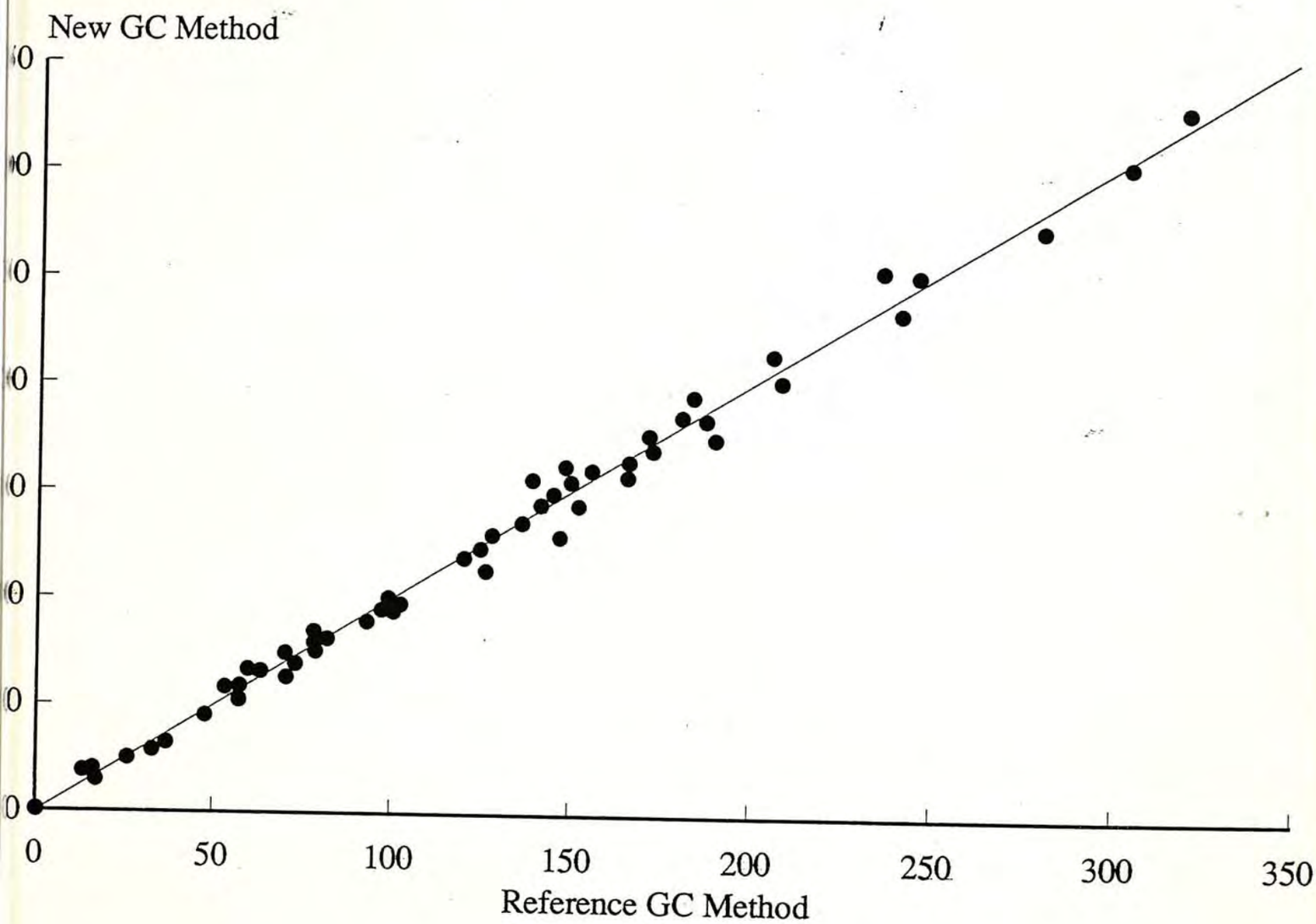
Table 9.

The calibration range and day-to-day variation (n=10) of the GC assay for plasma PTH and NPTH.

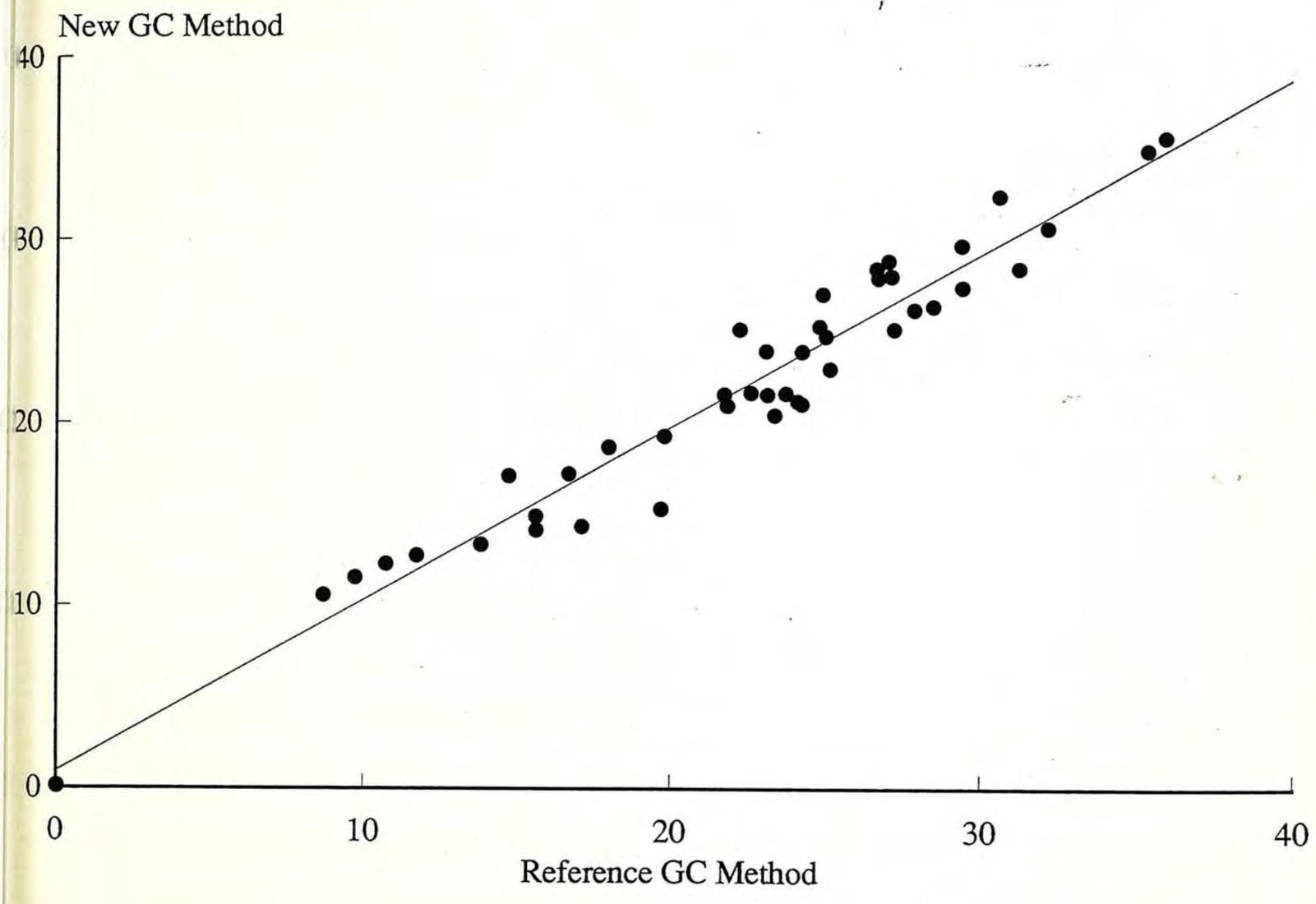
Conc. (ng/ml)	Peak height ratio ± S.D. (% C.V.)	
	PTH	NPTH
10	0.154±0.010 (6.5)	0.053±0.003 (5.7)
20	0.306±0.013 (4.2)	0.112±0.007 (6.3)
50	0.647±0.031 (4.8)	0.213±0.008 (3.8)
100	1.470±0.098 (6.7)	0.449±0.021 (4.7)
250	3.551±0.172 (4.8)	1.126±0.073 (6.5)
500	6.602±0.126 (1.9)	2.280±0.105 (4.6)
Correlation Coef.	0.9992	0.9998
Calibration graph	y = 0.0132x+0.0682	y = 0.00454x+0.00148
Batch standard at 0.1μg/ml (n=30)	1.451±0.081 (5.9)	0.454±0.032 (7.0)

Fig. 14a. **A comparison of the PTH results obtained by the described GC method (y-axis) and a reference GC method (x-axis).**



units?

Fig. 14b. A comparison of the NPTH results obtained by the described GC method (y-axis) and a reference GC method (x-axis).



4.3.3. Pharmacokinetics of PTH and NPTH in Chinese patients after intramuscular (i.m.) dosing

Figure 15 illustrated the mean plasma concentration-time curves of PTH and NPTH of the five Chinese patients after receiving a single intramuscular dose of pethidine. The individual plasma concentrations of PTH and NPTH were tabulated in Table A9 and A10 (p.192-193). The estimated minimum lethal dose of PTH is approximately 1g. Toxic effects (106) are usually associated with plasma concentrations of PTH and NPTH greater than 2000 and 700 ng/ml respectively. The maximum plasma concentrations among the five patients was found to be 326 ng/ml for PTH and 35.3 ng/ml for NPTH which were well below the toxic level. Non-compartmental analysis showed that the mean peak plasma concentration, the mean time for maximum absorption and the mean elimination half-life of PTH was 246.4±65.9 ng/ml, 0.73±0.35 hr, and 7.88±2.01 hrs respectively. Whereas NPTH appeared in the plasma from 0.5 to 1 hour and the mean peak plasma concentration was 28.8±4.38 ng/ml. The mean elimination half-life of NPTH (39.6±19.3hrs) was significantly greater than PTH. Table 10 summarized the mean pharmacokinetic data of PTH and NPTH of the five Chinese patients, whereas the pharmacokinetic data of each patient was shown in Table A11 and A12 (p.194-195).

Fig. 15. Mean plasma levels of pethidine (●) and norpethidine (♦) at various time interval after intramuscular administration of pethidine (1 mg/kg) to five Chinese patients.

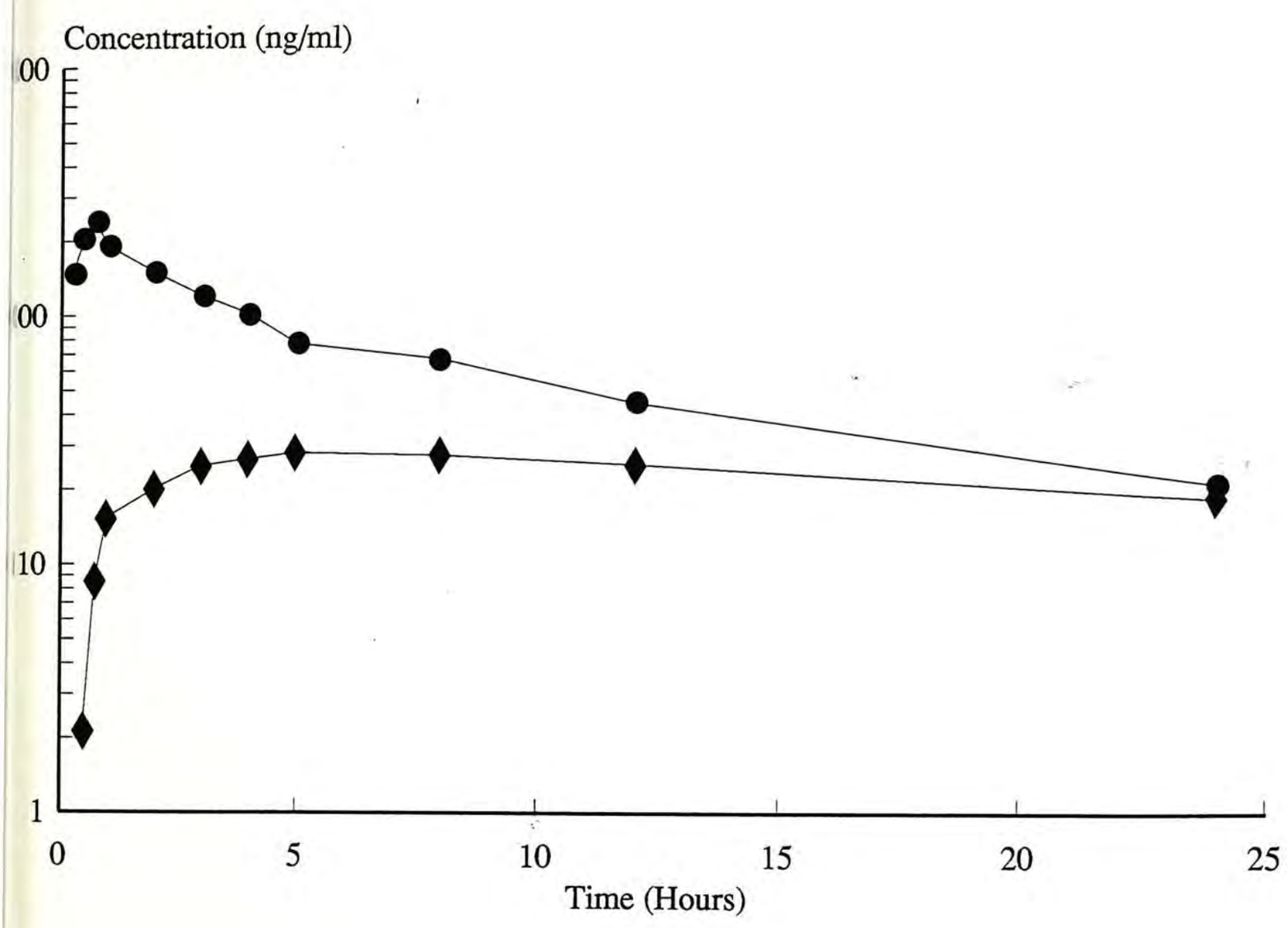


Table 10.

A summary of mean phamacokinetic data for PTH and NPTH after administrating 1 mg/kg PTH to five Chinese patients.

Phamacokinetic parameters	Pethidine (±S.D.)	Norpethidine (±S.D.)
$AUC_{0-\infty}$ (nghr/ml)	1810±442.7	1731±856
Elimination $t_{1/2}$ (hr.)	7.88±2.01	39.6±19.3
Tmax (hr.)	0.73±0.35	6.00±1.41
Cmax (ng/ml)	246.4±65.9	28.8±4.38
Clp (ml/kgmin)	9.69±2.52	-----
Vss (L/kg)	6.54±1.69	-----

4.4. DISCUSSION

The sensitivity of the majority of the established method based on gas chromatography with FID without prior derivatization (21,96-97,103) was low and did not enable the determination of NPTH in the plasma. The reported GC-MS assays were extremely sensitive (down to 0.5 ng/ml) and selective but these methods increased the cost of a routine analysis. As far as derivatization is concerned, PTH and NPTH often form the same derivative which must be separated by partition chromatography in a simple column separation before derivatization. Thus the proposed method was relatively simple and the sensitivity (5 and 10 ng/ml for PTH and NPTH respectively) was good enough to measure the compounds of interest in the plasma samples.

OV-17 was a classical GC column used in the determination of these narcotic analgesic drugs in the plasma (21,91-92,96-101). As observed by other workers (102-103), we found that the chromatographic performance of OV 17 was good for PTH but fairly poor for NPTH. The tailing NPTH peak could reduce the sensitivity of the whole assay and that might partly explain why most methods omitting the derivatization step were comparatively insensitive. Owing to the unsatisfactory detection limit of the countercheck method (103), the experimental condition of that method was slightly modified in such a way that analysis was carried out by using a NPD instead of a FID; the lowest detection limit was increased from 25 ng/ml using 5 ml sample to 10 ng/ml using a 1 ml sample.

Studies in patients following abdominal surgery suggested that blood PTH concentrations of 500-700 ng/ml were required for analgesia (107-108).

The concentration-analgesic respond relationship for PTH was extremely steep and dosage regimens of PTH which produce variable or fluctuating blood concentrations can be expected to achieve inconsistent relief of pain. But this regimen failed to take into account the pain tolerance when a patient-controlled analgesic therapy model in 20 patients, who received self-administered small intraveneous small doses of PTH, was studied (109). The maximum plasma concentrations ranged from 132 to 892 ng/ml and of the 20 patients, 19 obtained subjectively satisfactory analgesia. In our small group, the PTH concentrations were well below the recommended levels (107-108), but none of the patients suffered inadequate analgesia. The mean elimination half-life ($t_{1/2}$) of PTH was 7.88±2.01 hrs which was about 2 times higher than the two previously reported values (110-111), but was in agreement with the current findings (90). Since irregularities in plasma concentration in individual subject occurred (108,112), we expected a significant different $t_{1/2}$ in one patient within the small group could result in a significant change in the mean value. However the mean $t_{1/2}$ of the five Chinese patients fell within the normal expected range (3-8 hrs).

PTH was readily absorbed after intramuscular administration and peak plasma concentrations were observed between 1 to 2 hrs which were similar to the data obtained in oral route (112). The mean Vss of PTH was 6.54±1.69 L/kg suggested that this drug is extensively distributed into the tissues and this obtained Vss value was higher than the literature value (4.71 L/kg) (110). Although the mean concentrations of PTH in the plasma were higher than that of NPTH, their AUC values were similar. This indicated that

NPTH was eliminated slowly in the plasma as indicated by its long t1/2. From the studies of Stambaugh and Wainer (111), the elimination half life of NPTH was estimated from the rate of its excretion in urine, was around 12 hours and was dependent on the urine pH. But we have a surprisingly long half-life among the patients. NPTH was detected in the plasma from 0.25 to 0.75 hr after PTH was administered and it reached its maximum concentration at about 6 hrs. The half-life of NPTH (39.6 hrs) was relatively longer than the usual sampling time of 24 hours. Although it was not suitable to extrapolate the curve to calculate this t1/2 from the last three or four points in the the elimination phase, it was difficult to collect blood samples at various intervals over 24 hours so that the values determined were an estimation. We expected that if this metabolite was given to healthy volunteers, one might determine this pharmacokinetic data more precisely and the other parameters such as clearance and volume of distribution which we could not found in our study, could be compared with its parent drug, PTH.

4.5. CONCLUSION

A sensitive and accurate gas-liquid chromatographic method was described to determine pethidine and norpethidine in plasma of Chinese patients who received a single dose (1 mg/kg of total body weight) of pethidine intramuscularly. The chromatograph consisted of a 3% W/W SP 2250 on Chromosorb W glass column linked to a nitrogen detector. Sample preparation was simple, being based on a single extraction of basified plasma samples with n-hexane. The operating time of the assay was short and was suitable for routine pharmacokinetic studies of both drugs. The method was sensitive down to 5 and 10 ng/ml for pethidine and norpethidine respectively from 1 ml plasma sample. Linearity was observed in the range of 10 to 500 ng/ml. In a preliminary study on 5 Chinese patients who received pethidine treatment, the mean±S.D. peak plasma concentration, time for maximum absorption and elimination half-life were 246±66ng/ml, 0.73±0.35 hr and 7.88±2.01hrs respectively. The metabolite appeared in the plasma from 0.5 to 1 hour and the mean peak plasma concentration was 28.8±4.38 ng/ml and its elimination half-life was significantly greater than the parent compound, pethidine.

CHAPTER 5

DETERMINATION OF PETHIDINE AND ITS MAJOR METABOLITES IN HUMAN URINE BY GAS-LIQUID CHROMATOGRAPHY

5.1. INTRODUCTION

As mentioned in the previous Chapter, major metabolites of pethidine (PTH) such as norpethidine (NPTH), pethidinic and norpethidinic acids (PTA and NPTA) and conjugates of pethidinic and norpethidinic acids (PTC and NPTC) were biotransformed after PTH was metabolized in the liver by N-demethylation, hydrolysis and conjugation. While there were well established methods for the analysis of PTH and NPTH such as spectroscopy (83-86), ion-selective electrodes (88) and chromatography (89-102), the measurements of other metabolites were very limited. The reasons were either due to greater difficulty in extracting these compounds via organic solvents or their amounts in the biological samples were very low compared with PTH and NPTH. For instance, the solubilities of PTA and NPTA in aqueous medium were rather high and therefore these two compounds required to undergo esterification (113) or extractive alkylation (114) prior to chromatography. The measurements of minor metabolites like pethidine N-oxide (115) and N-hydroxynorpethidine (82) were performed by complicated GC-MS selected ion monitoring. To our recent knowledge we are not aware of any information in the literature concerning the analysis of PTH together with all its major metabolites in biological materials. A successful method of

this type could help us to further understand the metabolic routes of PTH among individuals. To achieve this purpose, we initially attempted to follow the procedure of the simultaneous HPLC assay of p-aminobenzoic acid and its conjugates (116) in urine samples by direct injection after centrifugation. We found that this method was not satisfactory because using UV detection at low wavelength (around 205 nm) the analytical peaks of PTA, NPTA, PTC and NPTC were subject to the interferences from the urinary endogenous substances, and they were not resolved from the enhanced interfering peaks even when ion-pair reagent (heptanesulphonic acid) was added. We now report a reliable GC method to determine PTH and the above major metabolites in human urine of eight Chinese patients who were receiving a single intramuscular dose of pethidine for post-operative analgesia. The method was based on the modification of our recently developed GC assay for monitoring the plasma levels of PTH and NPTH (Chapter 4).

5.2. EXPERIMENTAL

5.2.1. Materials and apparatus

Pethidine hydrochloride was obtained from May and Baker Ltd. (Dagenham, England), norpethidine hydrochloride from Sterling Winthrop Research Institute (New York, USA), chlorpheniramine hydrochloride from the Astra Pharmaceutical Production AB (Sweden) and β-glucuronidase (from *Helix pomatia*) from Sigma (St. Louis, MO, USA). Absolute ethanol, acetic acid, diethyl ether, methanol and sodium acetate (all of analytical grade) were purchased from E. Merck (Darmstadt, FRG). The following glassware was used: 15-ml capacity centrifuge tubes with well fitted screw caps containing PTEE linings, and 15-ml capacity Quick-fit glass tubes with tapered base of 50 µl. All glassware was cleaned and silanized with 3% HMDS in chloroform before use. Stock solutions containing 1 mg/ml PTH, NPTH, PTA and NPTA were prepared in methanol and stored at -20°C before use. The freeze drier (Savant Instruments, New York) consisted of a speed vacuum concentrator (SYC-100H), a refrigerated condensation trap (RT-100A) and a rotary vacuum pump (VP 100).

5.2.2. Gas chromatograph

The gas chromatograph was a Varian Model 6000 equipped with a nitrogen-phosphorus detector. A coiled glass column (2.4m x 2mm i.d., 6mm o.d. Supelco, Inc., Bellefonte, U.S.A.) was packed with 3% W/W SP2250 on Chromosorb W, 80-100 mesh. The column was silanized with 2 x 50 µl 3%

HMDS and conditioned at 300°C overnight before use. The column temperature was maintained at 230°C and that of the detector and injector was at 260°C. The signal was recorded and displayed by a flat-bed recorder (Linseis, Model L6512). The gas flow rates were: nitrogen (carrier gas) at 30 ml/min, air at 175 ml/min and hydrogen at 4.5 ml/min.

5.2.3. Synthesis of PTA and NPTA

Pethidinic and norpethidinic acids were synthesized by alkaline hydrolysis of their corresponding esters, (PTH and NPTH respectively). A 0.1g quantities of PTH and NPTH were separately placed in a 25 ml round bottom flask filled with 0.2 ml of conc. NaOH (6M) and 4.8 ml of ethanol. The mixtures were refluxed for 2 hours. After cooling to room temperature, 1 ml of 6M HCl was added to convert the sodium salts to the corresponding acids and the precipitates (NaCl) were filtered off. The acids in the ethanolic layer were isolated and purified by recrystallization in absolute methanol. The percentage yield for PTA and NPTA were around 48 and 36% respectively. The purities of these two compounds were checked by thin layer chromatography (69) and the results were satisfactory.

5.2.4. Analytical procedures for assaying PTH and NPTH (assay I)

A flow diagram of the assay procedures is shown in Fig. 16. To 0.25 ml urine samples, 0.75 ml of acetate buffer (pH 5.4) was added to 15-ml centrifuge tubes. The tubes were incubated at 37°C in a water bath for 18 hours and then 20 µl of 5M sodium hydroxide solution, 5 µl of the internal

standard, chlorpheniramine hydrochloride (0.4 mg/ml) and 5 ml of freshly distilled diethyl ether were added to the mixtures. The tubes were mixed for 5 min using an automatic shaker and centrifuged to break up the emulsions. The aqueous layers were separated and reserved for assay II. The ether layers were transferred to 15-ml evaporating tubes and were evaporated under a gentle stream of nitrogen at 45°C. The residues were redissolved in 10 µl of methanol and 1-µl aliquots were taken for analysis.

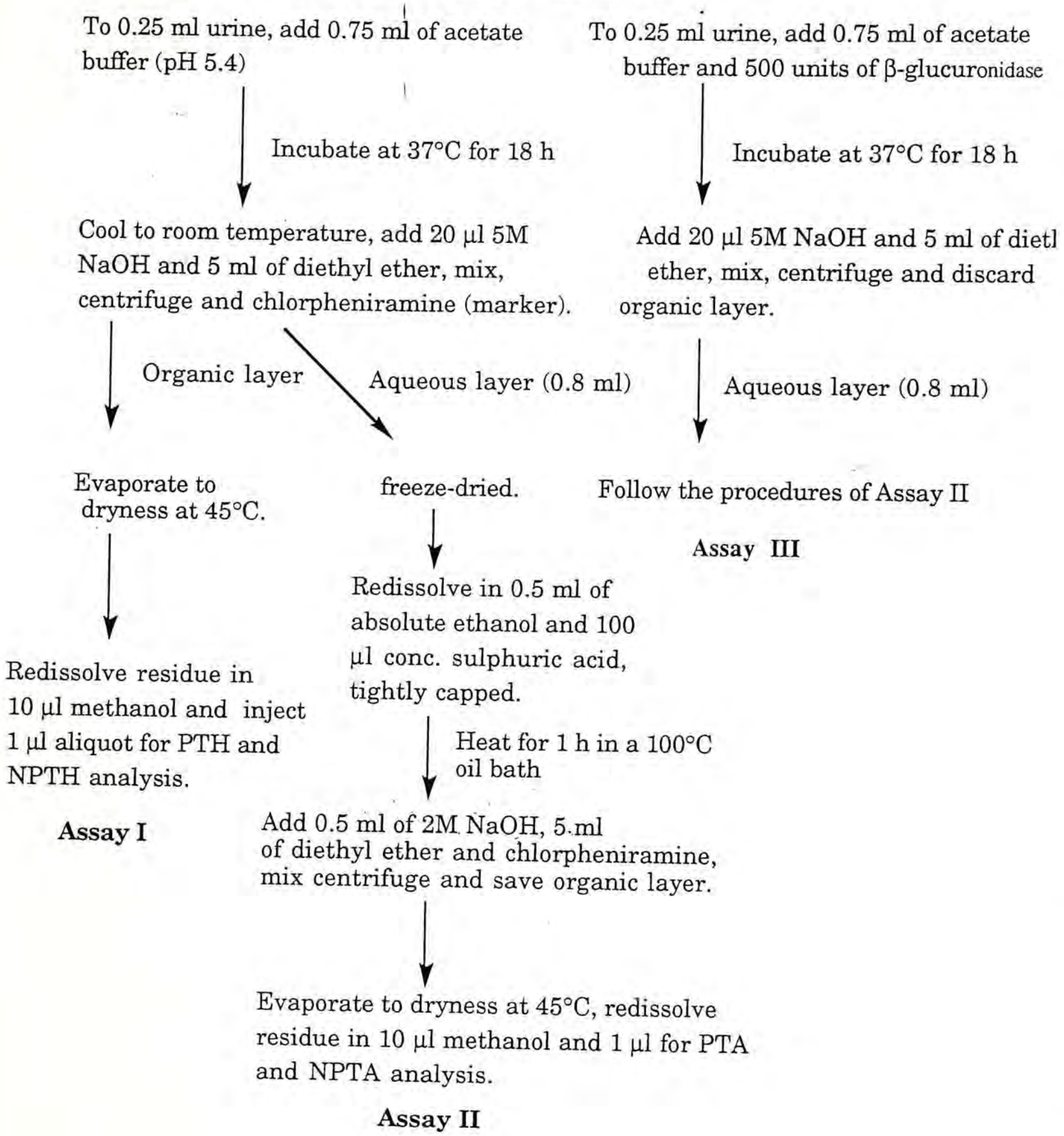
5.2.5. Analytical procedures for assaying PTA and NPTA (assay II)

The aqueous phases (0.8 ml) of the from assay I above were evaporated separately to dryness by freeze drying using the Savant freeze-dryer. The residues were each dissolved in 0.5-ml absolute ethanol and transferred to 15-ml centrifuged tubes containing 100 µl of concentrated sulphuric acid. The tubes were then tightly capped and heated at 100°C in an oil bath for 1 hour. After the acid catalyzed hydrolysis, the tubes were cooled to room temperature and 0.5 ml of 2M NaOH was added to neutralize the acid in the mixture, followed by the addition of 5 µl of chlorpheniramine hydrochloride (0.4 mg/ml) and 5-ml diethyl ether. The rest of the procedures were identical to that of assay I.

5.2.6. Analytical procedures for assaying total PTA and NPTA (assay III)

The procedure was the same as assay II, except for an additional

Fig. 16. A flow diagram to show the analytical procedures of assaying PTH and its major metabolites in urine samples.



enzymic treatment before the acid hydrolysis. To 0.25-ml urine samples, 0.75-ml acetate buffer solution (pH 5.4) and 500 units of β-glucuronidase were added. The tubes were incubated at 37°C for 18 hours and the mixtures were mixed and centrifuged after adding 20 μl of 5 M sodium hydroxide solution and 5 ml of diethyl ether. The aqueous layers (0.8-ml) were saved and the rest of the procedures were identical to that of assay II. The amount of PTC and NPTC were deduced from the differences between assays II and III.

5.2.7. Quantitation

Calibration graphs were constructed by spiking PTH, NPTH, PTA and NPTA into drug-free urine samples. Dilution of the stocks with methanol to prepare standards to cover the concentration range 0.4-6.4 μg/ml for PTH and NPTH and 0.8-12.8 μg/ml for PTA and NPTA. Between-day standards of concentration of 3.2 μg/ml were determined to obtain the between-batch variation of the assays. The calibration graph measurements were repeated six times during the course of study. The recoveries of PTH and NPTH after esterification of PTA and NPTA at 3.2 μg/ml in the urine samples were also determined.

5.3. RESULTS

5.3.1. Choice of extracting solvent

During the developing stages of the previous work (Section 4.3.1., Chapter 4), various commonly used solvents were investigated for recovering PTH and NPTH in plasma samples. The percent recovery was determined for each drug by comparing the peak heights of extracted plasma with the peak heights of methanolic standards at 0.1 μg/ml (external standard quantitation). It was shown that diethyl ether gave higher recoveries (>90%) but dirtier chromatograms (Table 7, Chapter 4). The interfering peaks in the chromatograms were due to the plasma endogenous materials and these substances were not found in the extract of urine samples, and therefore diethyl ether was considered as the suitable extraction solvent in this assay.

5.3.2. Recovery of the esterification of PTA and NPTA

The mean±S.D. conversion of PTA and NPTA to the corresponding ester at 3.2 μg/ml were 65.4±3.7% and 63.7±2.6% respectively. The percentages of conversion of PTC and NPTC to PTA and NPTA respectively during the acid catalyzed hydrolysis were also accessed. This was achieved by saving the aqueous layers (of those samples without treating β-glucuronidase after hydrolysis and extraction with ether, ie, assay II) and neutralized with appropriate volumes of 5M sodium hydroxide. The aqueous mixtures were adjusted with buffer to pH 5.4, incubated and then followed the procedures of assay III. These results (amounts of PTC and NPTC) were compared with the

values obtained from the difference between assays II and III. It was found that a fraction of PTC and NPTC were hydrolyzed to their respective acids, but the quantities were usually not more than 7% (n=6). In spite of this small percentage conversion, the present method still provides a good estimation of these compounds in patients' urine samples.

5.3.3. Performance of the GC procedure

The analytical peaks of PTH, NPTH and chlorpheniramine (internal standard) were well resolved with good symmetry (Fig. 17) and the retention times were 3.3, 4.5 and 7.5 min respectively. After enzyme treatment and esterification in acidic medium, there was an increase in the peak heights of PTH and NPTH (Fig. 18), indicating that PTC and NPTC were converted to the corresponding acids accordingly. The performance of the GC was summarized in Tables 11 and 12. It was found that the calibration graphs were linear over the range of 0.4-6.4 μg/ml for PTH and NPTH (r=0.9998 and 0.9994 respectively) and 0.8-12.8 μg/ml for PTA and NPTA (r=0.9994 and 0.9988 respectively). The between-day coefficients of variation at 3.2 μg/ml were 4.02%, 4.21%, 5.11% and 6.32% respectively. The corresponding lowest detection limits of PTH and NPTH were 0.01 μg/ml and of PTA and NPTA were 0.02 μg/ml. These values were sufficiently sensitive in the quantitative determination of their urinary concentrations within 24 hours after administration.

Fig. 17. Chromatograms showing (left) analytical peaks of PTH (1) at 0.71 μg/ml, NPTH (2) at 2.8 μg/ml in patient's urine sample spiked with (3) chlorpheniramine, the internal standard and (right) blank urine.

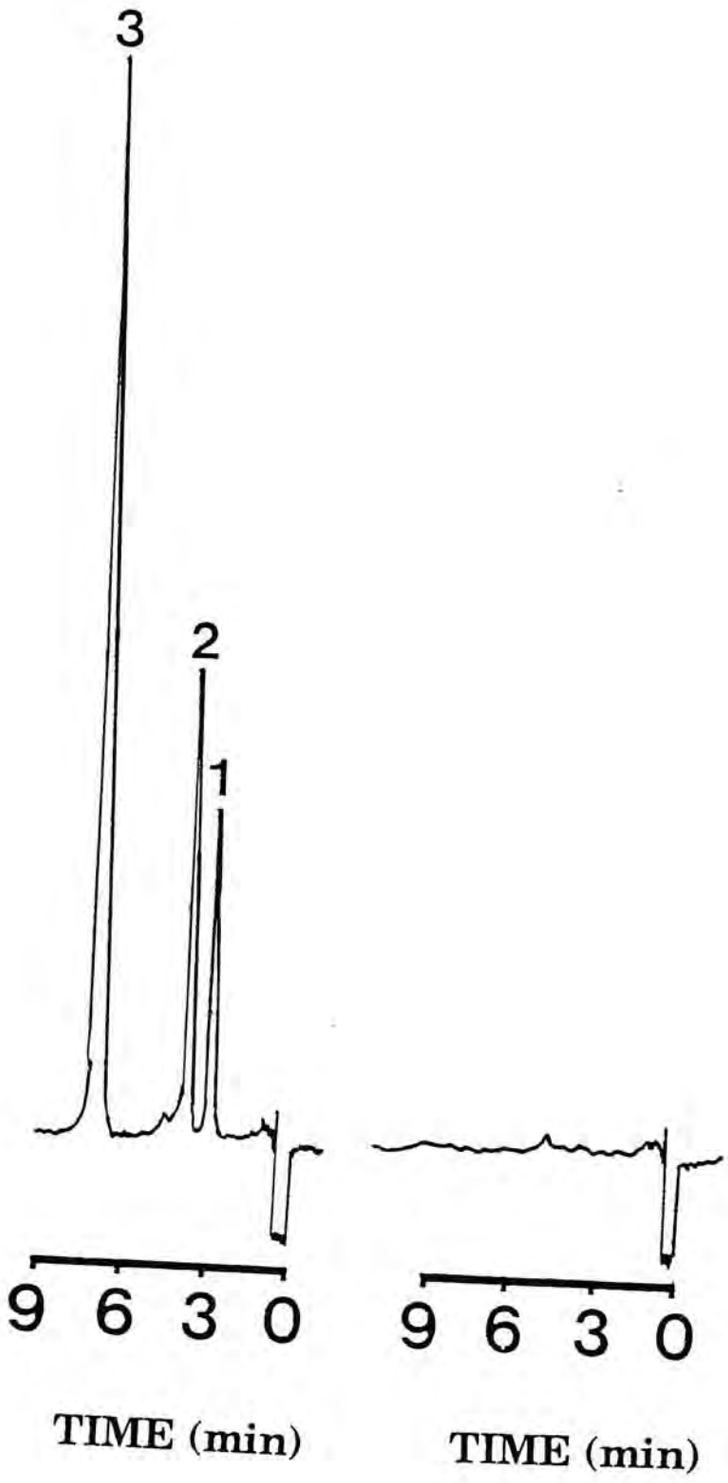


Fig. 18. Chromatograms showing (left) patient's urine sample after acid catalyzed esterification, (middle) patient's sample after both acid catalyzed and β-glucuronidase hydrolysis. Note that the increase in height of peaks 1 and 2, and (right) blank urine.

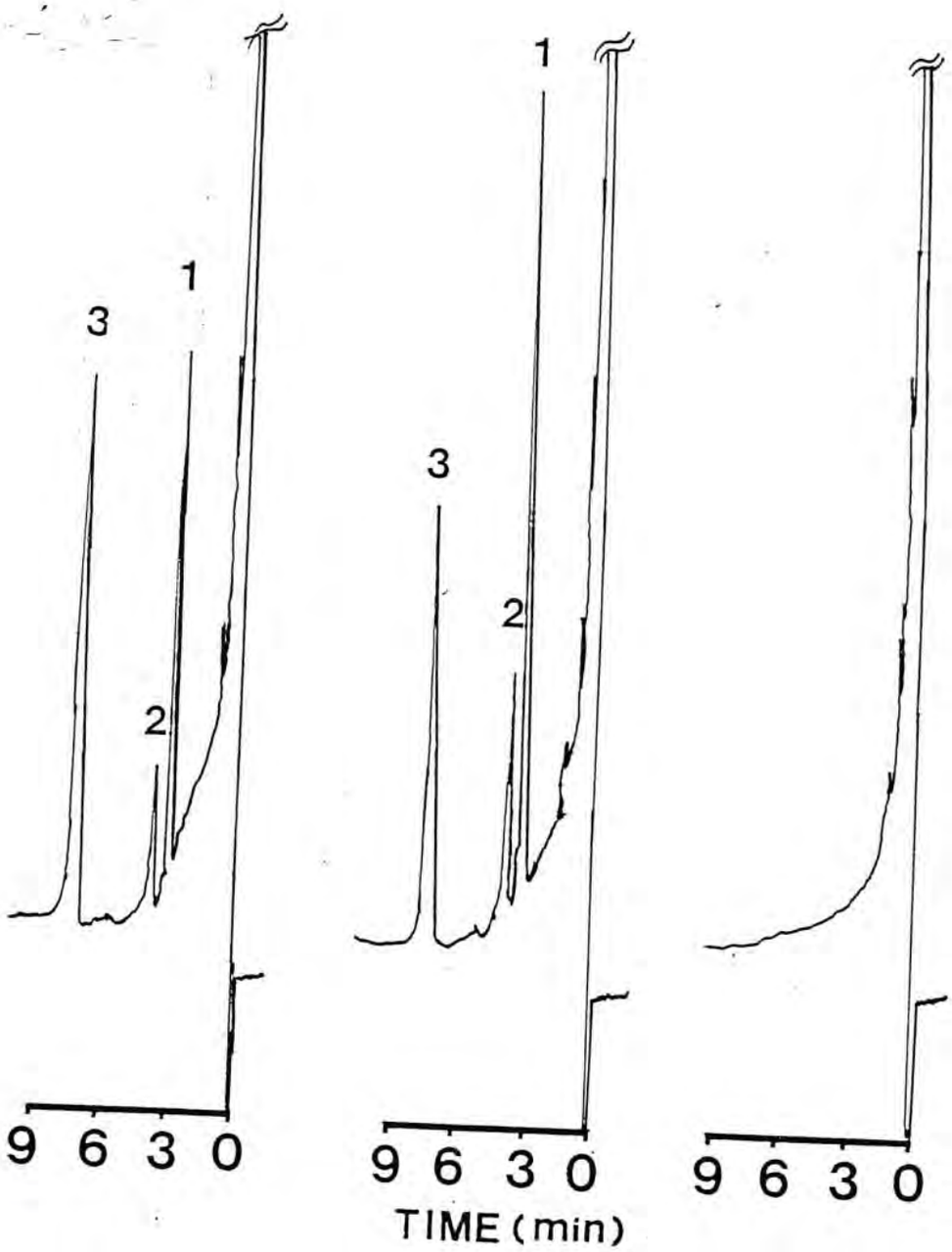


Table 11.

Calibration and precision of PTH and NPTH assay (n=6).

Concentration (μg/ml)	Peak height ratio ± S.D. (C.V.%)	
	Pethidine	Norpethidine
0.4	0.165±0.00849 (5.15)	0.0673±0.00408 (6.06)
0.8	0.347±0.0178 (5.13)	0.142±0.00738 (5.20)
1.6	0.624±0.0274 (4.39)	0.267±0.0112 (4.19)
3.2	1.28±0.0448 (3.50)	0.541±0.0211 (3.90)
4.8	1.96±0.0820 (4.18)	0.813±0.0273 (3.36)
6.4	2.62±0.107 (4.08)	1.13±0.0295 (2.61)
Batch standard at 3.2 μg/ml (n=10)	1.29±0.0519 (4.02)	0.537±0.0226 (4.21)
Calibration graph	y=0.409x-0.00513 (r=0.9998)	y=0.175x-0.00786 (r=0.9994)

Table 12.

Calibration and precision of PTA and NPTA assay (n=6).

Concentration (μg/ml)	Peak height ratio ± S.D. (C.V.%)	
	Pethidinic acid	Norpethidinic acid
0.8	0.186±0.00926 (4.98)	0.0795±0.00548 (6.89)
1.6	0.317±0.0873 (2.78)	0.164±0.00100 (6.10)
3.2	0.802±0.0413 (5.15)	0.339±0.0203 (5.99)
4.8	1.25±0.0745 (5.96)	0.462±0.0196 (4.24)
6.4	1.78±0.0667 (5.21)	0.627±0.0245 (3.91)
12.8	3.52±0.141 (4.01)	1.35±0.0326 (2.41)
Batch standard at 3.2 μg/ml (n=10)	0.807±0.0412 (5.11)	0.327±0.0207 (6.32)
Calibration graph	y=0.283x-0.0860 (r=0.9994)	y=0.105x-0.0151 (r=0.9988)

5.3.4. Application of the assay

Eight Chinese patients who underwent either hernia repair or excision exostosis were given a single dose (1 mg/kg of the total body weight) of pethidine intramuscularly. Bulked urine samples were collected in plastic containers until 24 hours after the intramuscular dose, the pH values were measured and then immediately stored in a freezer at -20°C before analysis. Samples were assayed in duplicate in order to enhance the accuracy of the results. The amount of PTH and its metabolites were found to be vary considerably among the eight subjects (Table 14). The excretions of PTH, NPTH, PTA, NPTA, PTC and NPTA were between 0.97-15%, 2.0-6.0%, 12.9-30.1%, 4.18-17.2%, 11.4-20.4% and 4.7-10.6% respectively over 24 hours after dosing. The percentage of unchanged drug and N-demethylated metabolite, NPTH excreted were smaller than the other metabolites. The dose recovery of the patients were between 57.1 to 68.5%, with a mean±S.D. value of 61.6±5.14%.

Table 13.

Dose recovery of PTH and its metabolites over 24 hours in patients' urine.

Compound		Recovery (%)						
Patient	pH	PTH	NPTH	PTA	NPTA	PTC	NPTC	Total
1	5.98	5.71	3.82	18.4	9.32	14.7	5.66	57.6
2	5.15	15.0	4.46	13.6	8.02	17.1	7.65	65.8
3	6.26	0.968	3.79	14.6	17.2	20.4	9.26	66.2
4	5.39	13.3	2.04	12.9	8.09	11.4	10.6	58.3
5	6.46	5.68	4.39	19.6	13.2	17.5	8.11	68.5
6	5.77	3.72	5.37	26.4	4.18	12.8	4.65	57.1
7	5.63	6.64	6.00	15.5	7.69	14.2	4.88	54.9
8	6.07	1.94	4.77	30.1	5.06	12.6	9.72	64.2
	MEAN	6.62	4.33	18.9	9.10	15.1	7.57	61.6
	S.D.	5.05	1.19	6.29	4.26	3.02	2.28	5.14

5.4. DISCUSSION

The chromatograms of the urine extracts were good in all cases. The analytical peaks suffered no interferences and baseline separation was obtained in the determination of PTH and NPTH. The signal to noise ratio of the solvent front in the analyses of acids and conjugates were shown to be decreased (Fig 18), but it did not affect the measurements of the peak heights of drugs and marker peaks. Since the volumes of urine excreted by the patients during the 24 hours experimental period varied remarkably and a large difference in the concentrations of the drug and metabolites could be expected. Therefore dilution of the urine sample was required if the concentrations of any drug were outside the calibration range. The addition of internal standard, chlorpheniramine, in assay II and III was critical. It must be added after the neutralization of residue acid left after esterification, otherwise this internal standard would be destroyed by the acid.

The above GC method involved very tedious analytical procedure. The loss of analytes and incomplete conversion during the indirect sample treatments possibly reduced the sensitivity of the assay and the lengthy steps further questioned its usefulness in routine analysis. Therefore we believe a direct and simultaneous HPLC method to measure these compounds could compensate and improve those disadvantages. Before we developed the described GC assay, we were struggling hard for a useful HPLC determination but we did not succeed because of the physical properties of the analytes. Since PTH and NPTH are weak bases and PTA and NPTA are weak acids, it was difficult to resolve these peaks simultaneously by compromising

the chromatographic parameters like pH of the mobile phase, flow rate and types of column. The step to achieve such type of HPLC measurement was still dubious and without the presence of direct determination of the metabolites, our described GC method was a good alternative in providing a useful tool for measuring these compounds in the pethidine disposition study.

With the information obtained from the previous papers regarding the enzyme incubation period, we noticed that the reaction time for the enzymatic hydrolysis of conjugated metabolites at 37°C were usually long, for instances, 10 h for conjugated N-hydroxynorpethidine (82), 18 h for conjugated 2-hydroxyimipramine (117) and 24 h for morphine-6-glucuronide (118) . Therefore we suggested to use a reasonably long incubation time (18 h) so as to attain a complete enzymatic process.

The percentage yield in the esterification of PTH and NPTH to PTA and NPTA were about 65%. These comparatively low recoveries were mainly due to incomplete reaction (equilibrium existed in acid catalyzed esterification), degradation or possible adhesion of the molecules onto the screw cap linings during the course of the reaction. Although the recovery might be increased if the reaction was performed under reflux condition, the major advantage of using screw-capped tubes was that, a large number of samples could be conveniently determined at one time, which was definitely useful in routine anlaysis. The novelty of this analytical method was a negligible loss of PTC and NPTC after acid catalyzed esterification, which provided us an important key to estimate the amount of these compounds in the urine samples. The regioselective reaction was likely because of the bulky

group effect of the glucuronyl with respect to the ethyl group. The nucleophilic (EtO-) attack on the carbonyl carbon of PTA/NPTA was comparatively easier than ~~that~~ on that of PTC/NPTC. Therefore while the acids were almost exclusively converted to esters, only less than 7% of the glucuronides were converted to the same products.

The amounts of the drugs found in the urine varied among individuals (Table 13). The mean recoveries of PTH, NPTH, PTA, NPTA, PTC and NPTC were 6.62±5.05, 4.33±1.19, 18.9±6.29, 9.10±4.26, 15.1±3.02 and 7.57±2.28% respectively. The excretions of PTA and PTC were higher than the other metabolites; conversely, a small amounts (around 5%) of unchanged PTH and the major metabolite, NPTH, were excreted in the urine samples. Therefore it seemed that hydrolysis and subsequent conjugation of the acid were the dominant routes of PTH metabolism in the patients. The mean dose recovery (61.6±5.14%) was lower than 100% because the quantities of these compounds excreted beyond 24 hours and quantities of other metabolites such as pethidine N-oxide, 4-hydroxypethidine and N-hydroxynorpethidine were not included in this study. None of the patients suffered from urinary acidosis. The pH values (5.15-6.46) of the urine samples were found to be within the normal range. Since pethidine is a weakly basic drug (pKa=8.63), it was likely that patients with lower urine pH excreted (Table 13) PTH more readily and these observations correlated well with the previous findings (119). The speculation that Oriental subjects ~~were~~ are better demethylators than Caucasians subjects (120-121) could be further evaluated if ~~justified~~ more urine samples of different races ~~are~~ were studied using the described method.

5.5. CONCLUSION

Procedures based on GC were established to determine pethidine and its major metabolites in human urine. The chromatograph consisted of a glass column packed with 3% w/w SP2250 on Chromosorb W, 80-100 mesh, linked to a nitrogen phosphorus detector. Diethyl ether was used as the extraction solvent. Pethidinic and norpethidinic acids, and their conjugated metabolites after β-glucuronidase treatment were converted to pethidine and norpethidine by acid catalyzed esterification. The retention times of pethidine, norpethidine and chlorpheniramine (internal standard) were 3.3, 4.5 and 7.5 min, respectively. The calibration ranges of PTH/NPTH and PTA/NPTA were 0.4-6.4 and 0.8-12.8 μg/ml respectively and with overall % C.V. of less than 6.89% (n=8). The day-to-day variation at 3.2 μg/ml were between 4.02 to 6.32%. The lowest detection limits by using a 0.25 ml urine sample were 0.01 and 0.02 μg/ml for PTH/NPTH and PTA/NPTA respectively. The amount of unchanged drugs and metabolites excreted varied considerably among the subjects. The mean 24 hours urinary recoveries in eight patients of pethidine, norpethidine, pethidinic acid, norpethidinic acid, and glucuronides of pethidinic and norpethidinic acids were 6.62±5.05, 4.33±1.19, 18.9±6.29, 9.10±4.26, 15.1±3.02 and 7.57±2.28% respectively, indicating that the major metabolic pathways of pethidine in the eight patients were hydrolysis followed by conjugation. Over 60% of the dose was accounted for in 24 hours after intramuscular administration of 1 mg/kg of pethidine.

CHAPTER 6

PLASMA PROTEIN BINDING CHARACTERISATION OF PETHIDINE AND NORPETHIDINE

6.1. INTRDUCTION

The fate of many drugs in the body is greatly influenced by their binding to plasma proteins (122,123). Only the fraction of the drug in the blood stream that does not bind to plasma proteins can leave the circulation, distribute throughout the body and reach the sites of action. Thus the nonprotein bound (free) drug is the best species correlated with the pharmacologic properties and more closely related to the drug effect than the total (bound plus unbound) drug concentrations (124,125). However, almost all the pharmacokinetic studies and dosage adjustments in patients are based on the determination of total drug concentrations in the plasma. This is because of the greater ease in measurement of total concentrations and the use of total drug seems to cause no problem as the relationship between free and total drug concentration is normally constant within and between individuals (126). But it will not be the situation if the binding changes significantly which can alter the relationship between these two drug concentrations and make the interpretation of total drug concentrations more difficult. Factors such as disease states (127) and concomitant drug therapy (128) can alter the extent of drug binding and ends up with erroneous and misleading results. The binding of one drug (displaced agent) to plasma proteins can also be displaced

by another drug (displacing agent), and such type of drugs interaction has been demonstrated in a number of clinically-used drugs, for examples, the displacement of warfarin by anti-inflammatory agents (129). The metabolite of trichloroacetic acid, chloral hydrate (130), thiopental by halothane (131); and phenytoin (132) and diazapam (133) by enflurane. Although displacement of drug from its binding sites can only increase free drug concentration in the plasma transiently and has no subsequent effect on the pharmacological activities, it may give rise to changed pharmacokinetic characteristics such as a reduction in metabolism (134) and elimination half-life (126). Therefore protein binding can be treated as an important pharmacokinetic parameter and these studies should be useful in interpreting pharmacokinetic data in clinical practice. The purposes of our work are to determine the binding affinity of pethidine (PTH) and its N-demethylated metabolite, norpethidine (NPTH), to plasma proteins and various plasma protein components, and to examine which is the major plasma component involved in the protein binding of these two drugs.

6.2. EXPERIMENTAL

6.2.1. Chemicals and Materials

Pethidine hydrochloride was obtained from May and Baker Ltd. (Dagenham, UK) and norpethidine hydrochloride from Winthrop Research Institute (New York, USA). Plasma proteins were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Because the proteins from various animal species differ greatly in their drug binding properties from human and therefore all the proteins were of human origin, they included albumin (HSA) (Sigma A-8763), α_1-acid glycoprotein (AAG) (Sigma G-9885), β-lipoproteins (Sigma L-2139) and γ-globulin (Sigma HG-11). The dialysis buffer was Sφrensen's phosphate buffer made by mixing KH_2PO_4 (9.073 g/L) and $Na_2HPO_4.2H_2O$ (11.87 g/L) and the pH was adjusted to 7.4 by using either one of the two solutions. Spectrapor dialysis membrane (molecular weight cutoff of 12,000-14,000) was obtained from Spectrum Medical Industries Inc. (Los Angeles, USA) and DIANORM® apparatus was purchased from Diachema AG (Zurich, Switzerland).

6.2.2. Blood Sample Acquisition

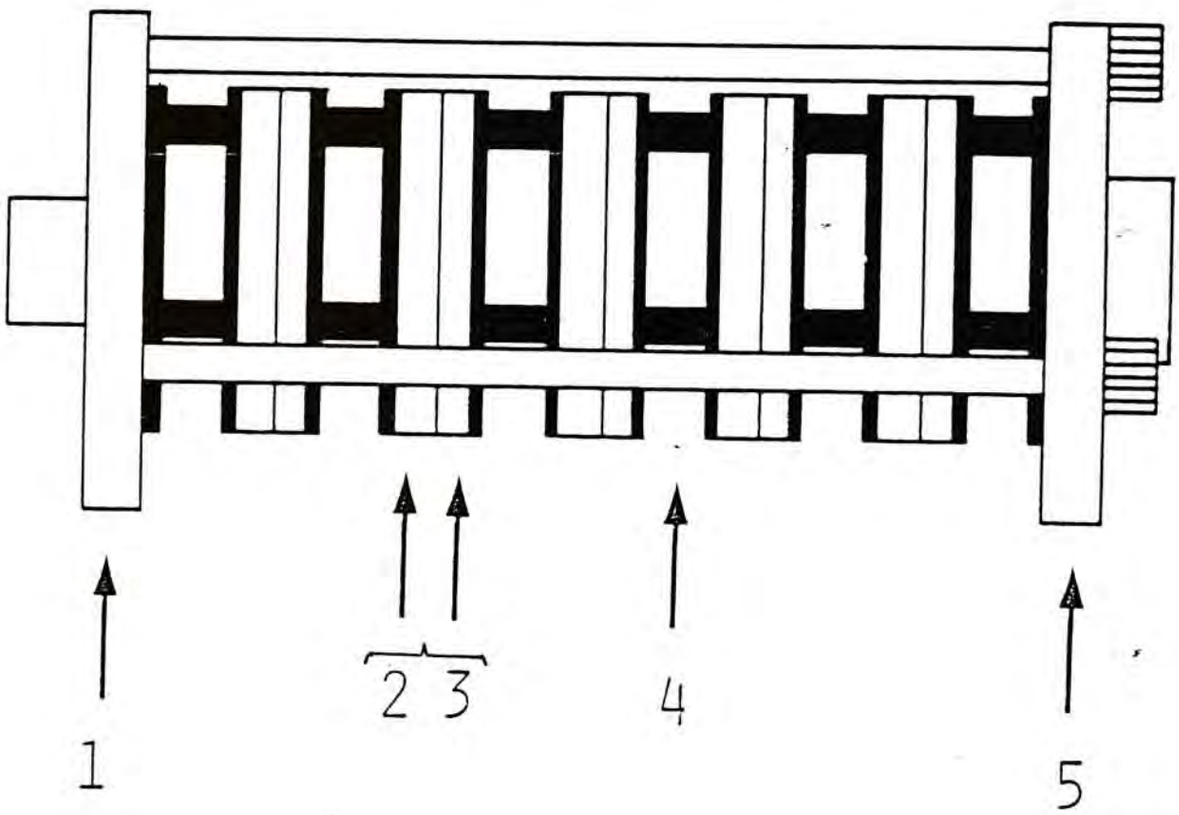
Fresh blood samples were obtained from two healthy, drug free and overnight-fasted male volunteers by antebrachial venipuncture. The samples obtained were immediately transferred into commercially available 10 ml plastic tubes containing ammonia heparin (Sarstedt, Numbrect, FRG) and centrifuged at 1000g for 20 min to yield plasma which was stored at -20°C

before use.

6.2.3. Equilibrium Dialysis and Plasma Protein Binding Study

Equilibrium dialysis was performed by means of a DIANORM® apparatus (Fig 19). Patients' plasma, drug-containing plasma or protein solutions (1 ml) were dialyzed against drug-free Sϕrensen's phosphate buffer (1 ml) in two Teflon dialysis chambers of a dialysis cell, separated by a dialysis membrane. Incubation of the cells was carried out at 37°C for 4 hours with a rotation speed of 12 r.p.m. To determine the binding of PTH and NPTH to isolated human plasma protein components, 0.5 and 0.1 μg/ml of PTH and NPTH were added to a range of protein concentrations. The concentration of various protein solutions were prepared to encompass the expected ranges in human plasma (HSA=30-60 g/L; AAG=0.5-15 g/L; β-lipoproteins= 5g/L and γ-globulin=12 g/L). The effect of drug concentrations on protein binding was determined within the range of 0.05-2.0 μg/ml for PTH and 0.025-1.0 μg/ml for NPTH respectively. Plasma concentrations of PTH and NPTH in the cell compartments were assayed after incubation by a recently established gas-liquid chromatographic method (Section 4.2.2., Chapter 4). Each experiment was repeated five times.

Fig. 19. A simplified diagram of a DIANORM apparatus containing five dialysing cells used for dialysis experiments. (1) Driving flange with guide rods, (2) Teflon cell base, (3) Teflon cell lid, (4) spring loaded cell spacers and (5) bearing flange secured with 3 knurled nuts.



N.B. A dialysing membrane is fixed in the position between (2) and (3).

6.2.4. Data Analysis and Interpretation

Protein binding was calculated according to Eq. 17, where Cp and Cbu are the drug concentrations in plasma and buffer after dialysis:

$$\frac{Cp - Cbu}{Cp} \times 100\% \ldots\ldots [17]$$

Scatchard analysis (135) (Eq. 18) was plotted to calculate the association constants and total binding capacities of PTH and NPTH to HSA and AAG.

$$\frac{B}{F} = -KB + nKPt \ldots\ldots [18]$$

where B and F are the bound and free molar concentrations of PTH or NPTH, K the association constant, n the binding capacity and Pt the protein concentration.

6.3. RESULTS

6.3.1. Protein binding at various concentrations of pethidine and norpethdine

The (mean±S.D.) plasma protein binding at 0.05, 0.1, 0.25, 0.5 and 1.0 mg/ml of PTH concentration were 72.1±2.81, 54.4±2.99, 49.0±3.30, 43.8±2.51 and 43.3±2.68% respectively; and at 0.025, 0.05, 0.1, 0.25 and 0.5 mg/ml of NPTH concentrations were 56.6±2.99, 45.4±3.25, 34.0±2.61, 29.4±2.33 and 26.7±3.88% respectively. A Scatchard plot was nonlinear for both PTH and NPTH (Fig 20 and 21), indicating that more than one type of macromolecules or more than one class of binding site on a single type of macromolecule may be involved in the binding. The B/F ratios of PTH and NPTH were almost linear (nonsaturable binding) at bound concentrations above 0.22 and 0.15 μmol/L respectively. The corresponding concentration binding sites may be calculated from the above figures by utilizing the relation nK = (B/F)/Pt, as 0.283 to 4.54 μM for PTH and 0.198 to 1.24 μM for NPTH. Since B/F ratio is linearly related to protein concentration, the total binding constants (nK) in HSA (45 g/L) and AAG (1 g/L) would be 1.12 to 3.79×10^3 M^{-1} and 30.5×10^3 to 10.3×10^4 M^{-1} respectively for PTH; and 14.6 to 52.4×10^3 M^{-1} and 12.6 to 52.4×10^3 M^{-1} respectively for NPTH.

6.3.2. Binding of pethidine and norpethidine with isolated plasma protein components at various concentrations

Protein concentration-dependent bindings of PTH and NPTH to HSA and AAG were shown in Fig 22 and 23 respectively. As the concentration of

Fig. 20. Bound/unbound (B/F) concentration ratio of pethidine as a function of bound pethidine concentration (mmole/L) in plasma.

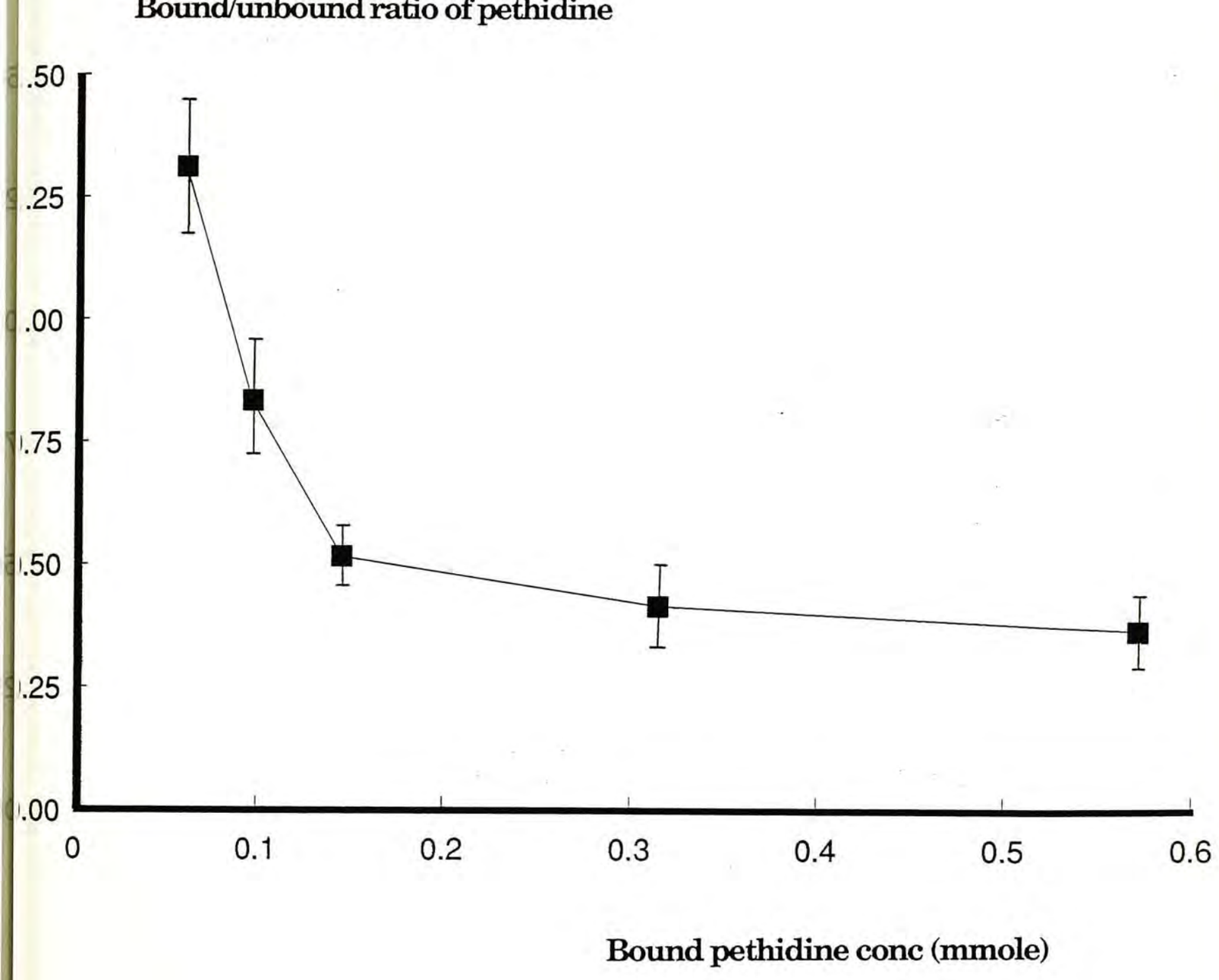


Fig. 21. Bound/unbound (B/F) concentration ratio of norpethidine as a function of bound norpethidine concentration (mmole/L) in plasma.

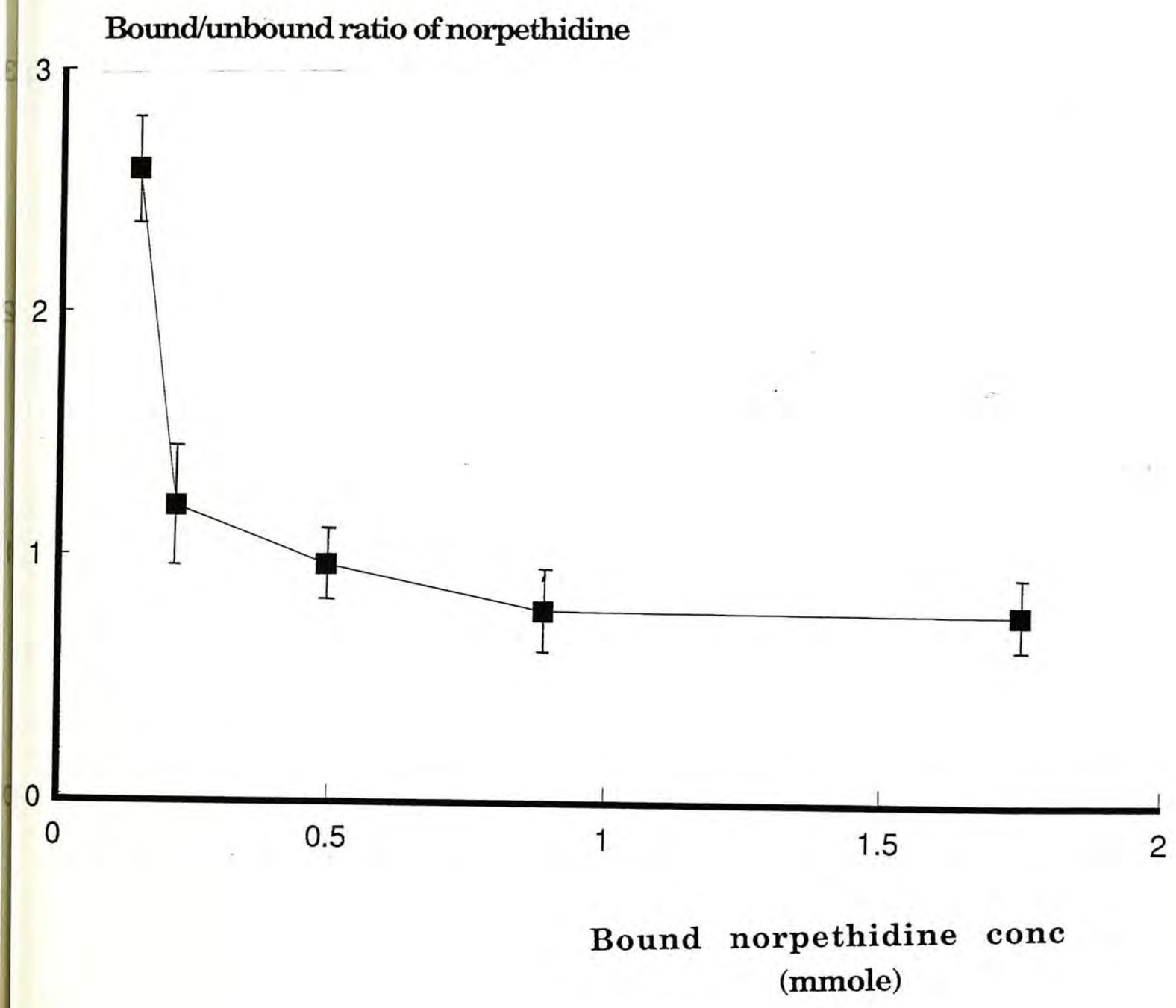


Fig. 22. Percentage bound pethidine at 0.5 μg/ml (■) and norpethidine at 0.1 μg/ml (♦) as a function of albumin (HSA) concentration (g/L).

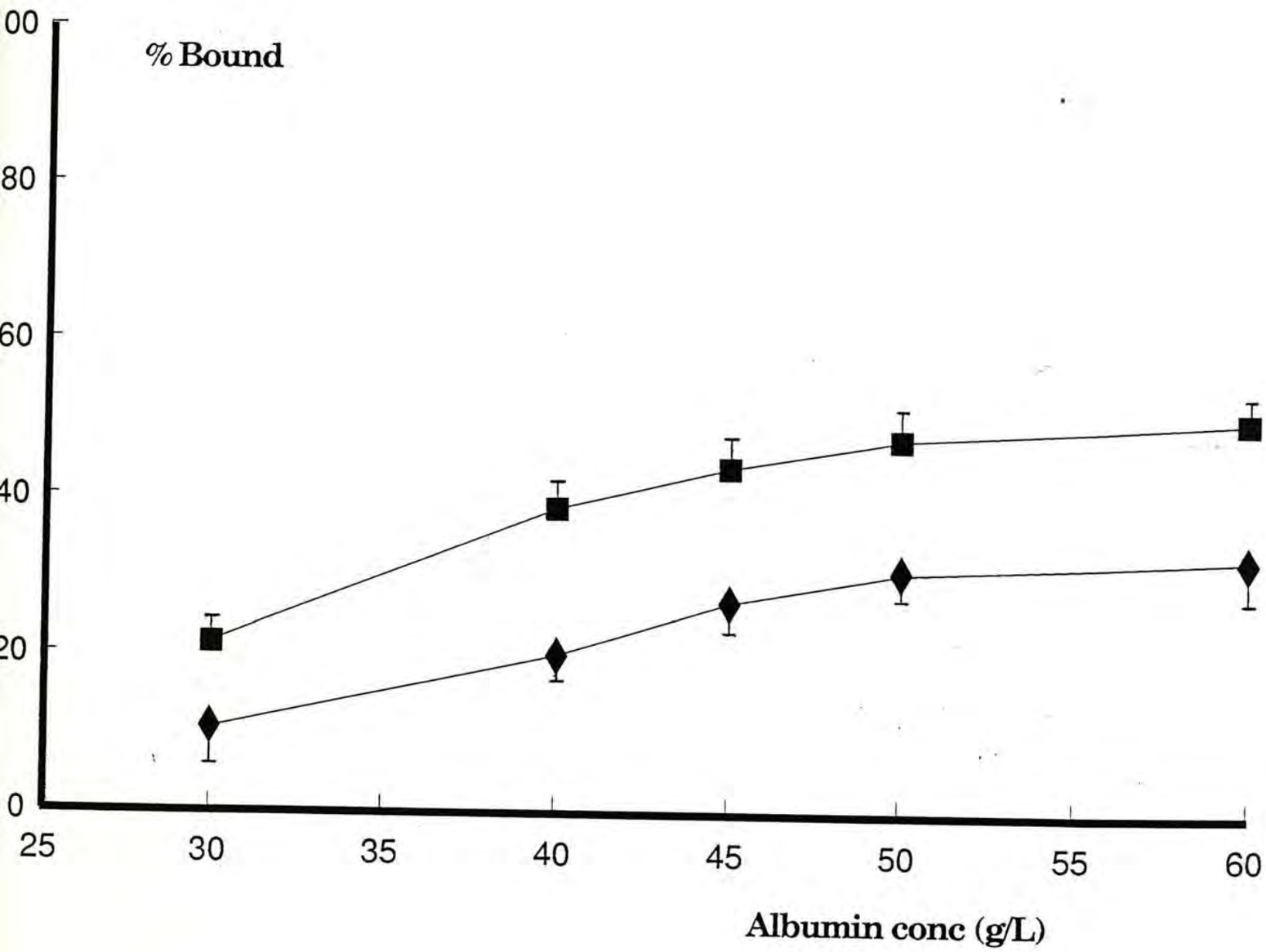
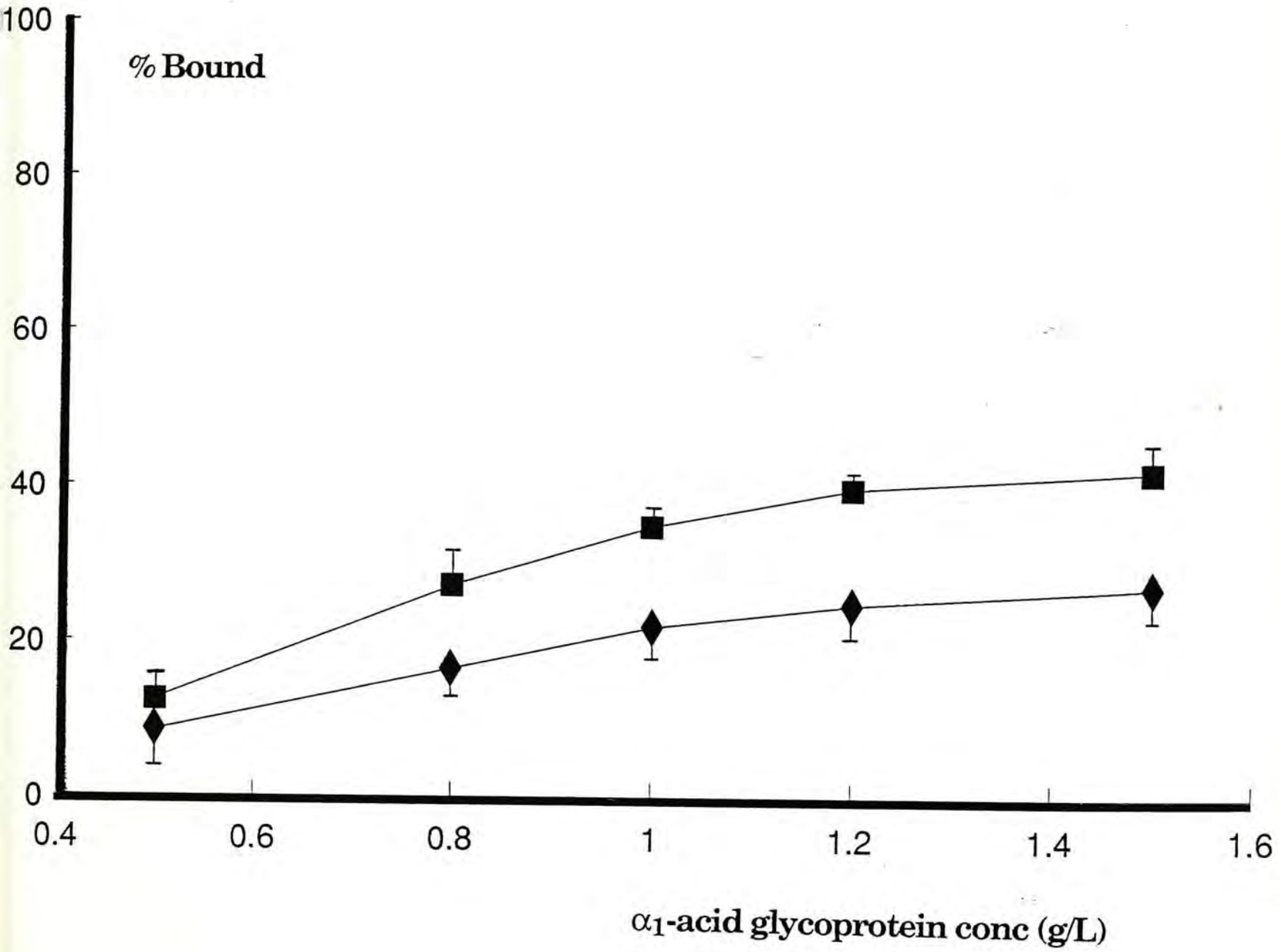


Fig. 23. Percentage bound pethidine at 0.5 μg/ml (■) and norpethidine at 0.1 μg/ml (♦) as a function of α_1-acid glycoprotein (AAG) concentration (g/L).



HSA and AAG in the buffer solution increased, the mean bound fraction of PTH and NPTH increased until a constant level was reached at 31% and 26% respectively. The degree of binding was also governed by the total drug concentration in the plasma. Fig 24 and 25 illustrated the (mean±S.D.) bound fraction of PTH at HSA (45 g/L) and AAG (1 g/L) fell from 80±3.0 to 44±3.4% and 78±3.6 to 31±1.7% respectively with increasing PTH concentration and NPTH fell from 69±4.5 to 18±1.9% and 60±4.9 to 17±2.3% respectively with increasing NPTH concentration. Data on the bindings of PTH and NPTH to plasma and physiological concentration of HSA, AAG, β-lipoproteins and γ-globulin were presented in Table 14. The bindings were determined within the therapeutic plasma level of PTH (0.5 μg/ml) and normal plasma level of NPTH (0.1 μg/ml) *in vivo* (106). The results represented the relative binding ability of PTH to plasma protein was in the descending order of HSA, AAG, β-lipoproteins and γ-globulin; and a similar trend was found in NPTH except that the degree of binding to β-lipoproteins was slightly greater than AAG. The drugs bind to HSA is comparable to those determined in plasma, further suggesting HSA is the major protein component involved in these bindings.

6.3.3. Displacement binding experiments

The displacement experiments (Table 15) showed that PTH and NPTH have no subsequent effect on displacing the plasma site with respect to one another. The mean percentage bound of PTH and NPTH were around 41

Fig. 24. Percentage bound pethidine to albumin (HSA) at 45 g/L (■) and α_1-acid glycoprotein (AAG) at 1 g/L (♦) as a function of pethidine concentration (μg/ml).

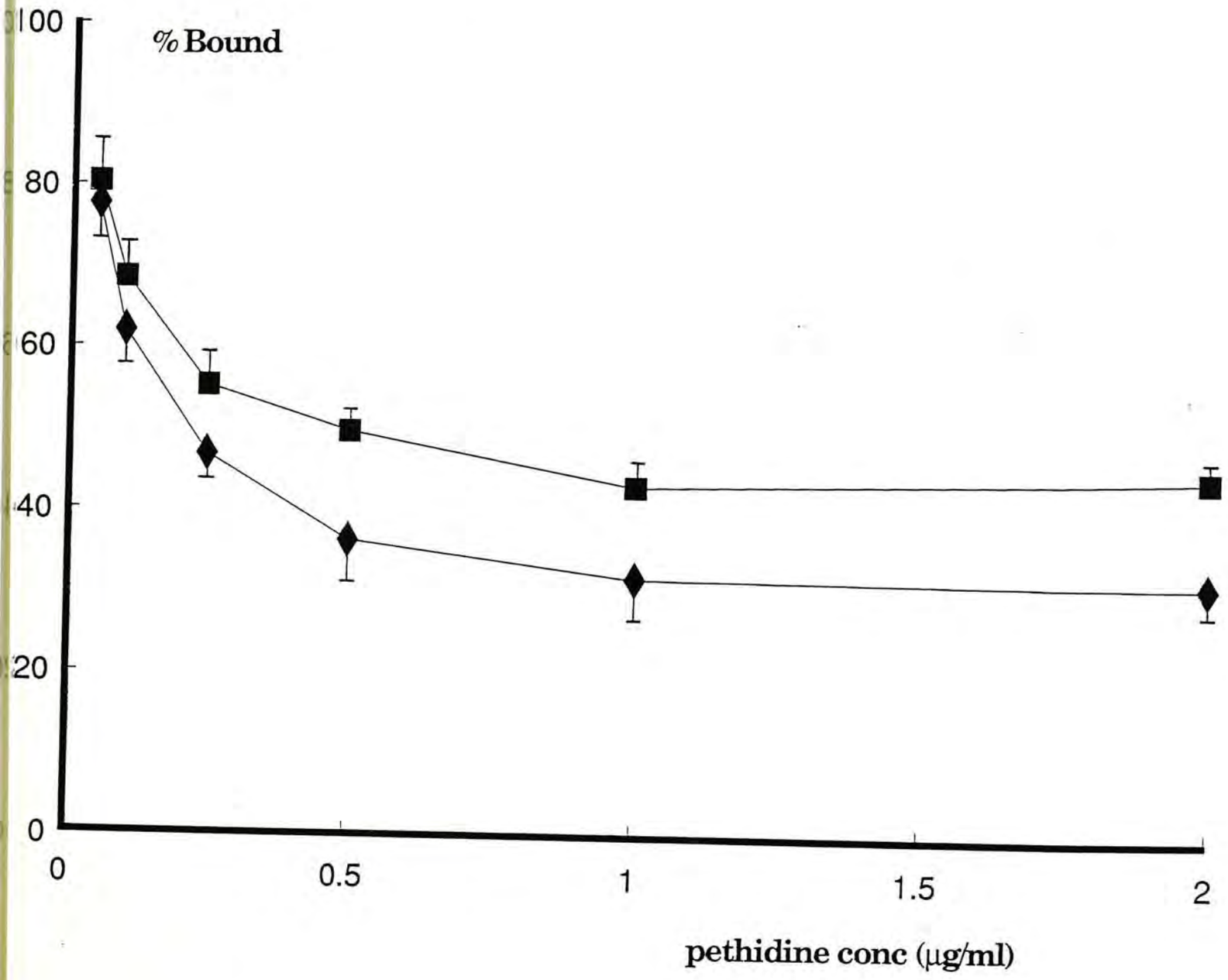


Fig. 25. Percentage bound norpethidine to albumin (HSA) at 45 g/L (■) and α_1-acid glycoprotein (AAG) at 1 g/L (♦) as a function of norpethidine concentration (μg/ml).

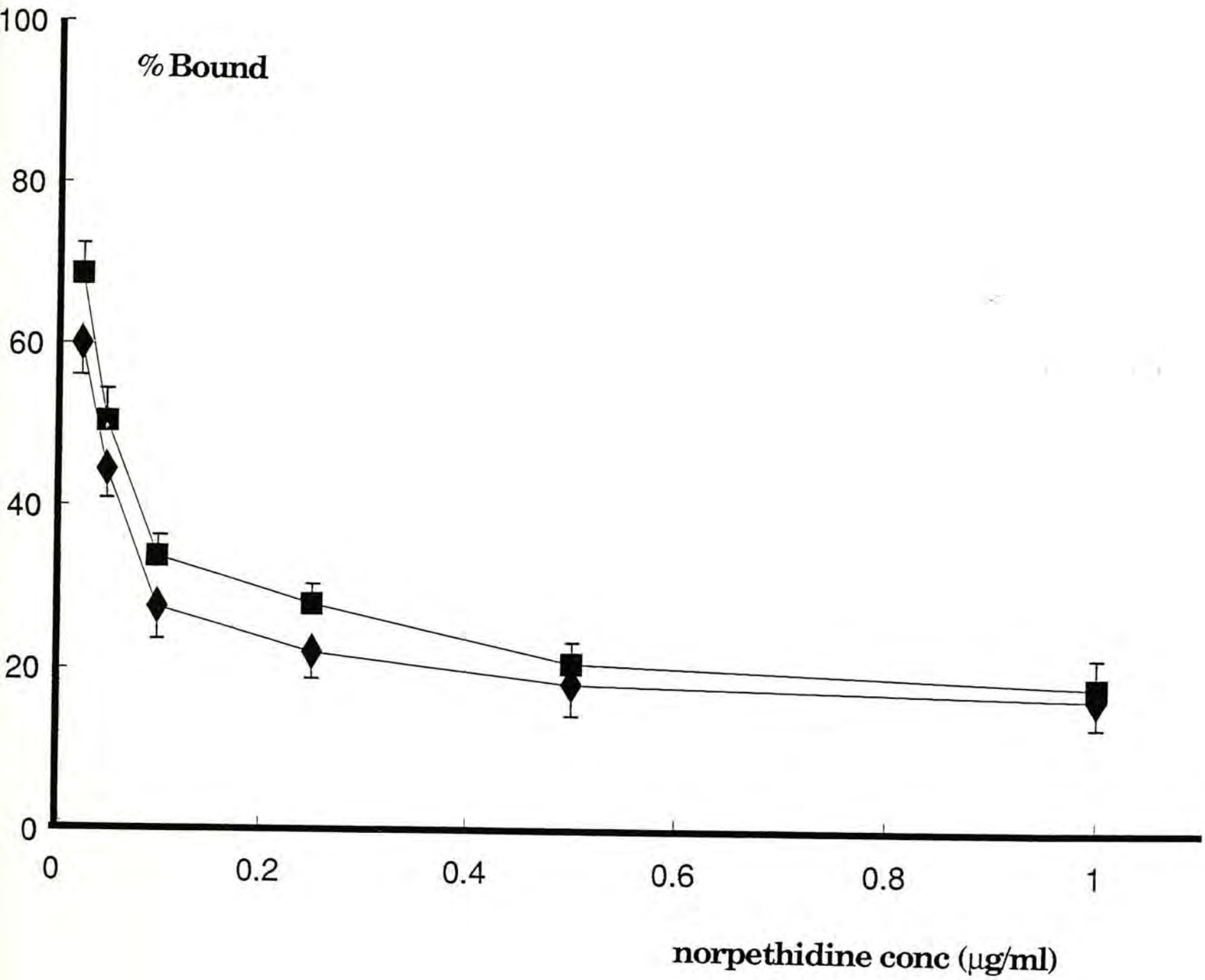


Table 14.

Protein binding of pethidine and norpethidine to human plasma and protein fractions at physiological concentration.

Protein	Protein conc. (g/L)	% binding (Mean±S.D.) PTH (0.5 μg/ml)	NPTH (0.1 μg/ml)
HSA	45	43.7±2.74	26.5±2.37
AAG	1	34.7±1.70	21.8±1.70
β-lipoproteins	5	29.0±2.95	22.7±3.08
γ-globulin	12	9.72±2.95	10.0±1.51
Plasma*	--	43.8±2.51	34.0±2.61

* Obtained from the two male healthy volunteers.

Table 15.

The displacement interactions on the plasma binding of pethidine and norpethidine.

NPTH conc. (μg/ml)	%PTH bound at 0.5μg/ml (Mean±S.D.)	PTH conc. (μg/ml)	%NPTH bound at 0.1μg/ml (Mean±S.D.)
0.01	41.47±2.66	0.05	34.35±2.50
0.02	38.51±2.38	0.10	32.43±2.24
0.05	41.61±2.97	0.20	31.44±2.61
0.10	40.36±4.53	0.50	33.54±2.31
0.20	43.08±4.59	1.0	33.08±2.88

and 33% respectively even when the concentration of the corresponding displacing agent has increased twenty folds.

6.4. DISCUSSION

Similar to most of the pharmacokinetic studies, we used heparin as the anticoagulant in blood collection. It was reported that a small amount of this agent in the blood could activate lipoprotein lipase and cause a decrease in plasma triglycerides and an increase in plasma fatty acids (136). Owing to the competition of these drugs and free fatty acids for the binding sites, these biochemical changes could reduce the binding characteristics of drugs such as propanolol (137) and quinidine (138). However such effect has not been shown in all the studies associated with pethidine protein binding.

The aspect of adequate methodology in the study of drug-protein interaction is usually the choice of assay procedure, device and materials. As mentioned in Chapter 1, dialysis, ultrafiltration, ultracentrifugation and gel filtration are the commonest employed methods but none of them has been considered sufficiently reliable and convenient to apply directly to the study (139) because of the difficulties in knowing if *in vitro* binding results are valid *in vivo*. Under most circumstances it appeared that equilibrium dialysis and ultrafiltration were equivalent (140), but another study showed the value of free fraction obtained was higher than ultrafiltration (141). In a recent study by Zhirkov *et al* (142) the usefulness of ultrafiltration was dubious and questionable because of severe non-specific adsorption on the membranes and the authors concluded that this method cannot substitute for the equilibrium dialysis in binding study; ultracentrifugation is very expensive and is not equipped in every

laboratory; gel filtration always requires large volume of protein solutions and it is not suitable for drugs with relatively low protein affinity too. Equilibrium dialysis has the disadvantages like Donnan effect (sample dilution), adsorption to the membrane materials and time consuming, but this technique is simple and can be easily performed at physiological temperature and is often regarded as the standard method in our laboratory.

During all stages of the experiments, the pH values and volume of plasma and buffer inside the cell compartments were not changed, and hence the suggested simultaneous correction for volume shift in dialysis (143) was not accounted for in the data analysis. The non-specific adsorption to dialysis membranes were minimized by soaking the membranes with drug solutions before use and then rinsed with buffer solution and distilled water. The dialysing time reported in the previous work was 2 hours (119) for PTH and 3 hours (70) for its similar analogue, phenoperidine. In our preliminary study for the investigation of optimum conditions, it was found that NPTH (4 hours) has a longer equilibrium time than PTH (3 hours) and a dialysing time of 4 hours was therefore chosen for all the experiments in this study.

The plasma protein bindings of PTH and NPTH determined were 43-72% and 27-57% over the drug concentration range tested. The mean (±S.D.) plasma PTH bound (54.4±2.99% at 100 ng/ml) agreed well with the reported mean value of 56.9% at 130 ngml (119). No information on the binding properties of NPTH was ~~acquired~~ found in the literature so that no

comparison with the obtained binding data can be made.

Although the attraction and specificity of orientation of a drug molecule toward its binding site on plasma proteins may be an electrostatic one, this interaction is reinforced by hydrogen bond, hydrophobic bond and dipole-induced dipole bonding. Many highly albumin-bound drugs are poorly soluble in water and for such drugs hydrophobic binding to hydrophobic sites on albumin is often important (144). The partition coefficients (69) between n-heptane and buffer (pH 7.4) of PTH and NPTH were 1.68 and 0.37 and we proposed that the higher binding affinity of the parent drug might be because of the greater hydrophobic property (in terms of K) associated with its structure. Unlike other drugs such as metoclopramide (145) and moricizine (146), the binding of both PTH and NPTH to plasma, HSA and AAG at physiological concentrations of proteins displayed obvious drug concentration dependency, reflecting saturable binding. This kind of dependency did not occur in β-lipoproteins and γ-globulin. Since the extent of drugs binding correlated with the amount of HSA and AAG available in the plasma, a variation in these proteins concentrations could reduce the consequent binding. This possibility was eliminated by the results of total plasma proteins determination (Table 18, Chapter 7) which showed that the plasma proteins levels of all patients were well within the normal concentration range (60 - 80 g/L).

Our study showed the major component involved in the binding of

both PTH and NPTH was mainly HSA and AAG while β-lipoproteins also contribute to these bindings but to a smaller extent compared with HSA. It is recognized that most of the binding of acidic drugs can be accounted for the association with HSA. Basic drugs do bind to HSA but also associate with other plasma proteins, usually AAG. Therefore the binding results of PTH and NPTH displayed a typical binding characteristics of weakly basic drugs.

If a drug competitively displaces another drug, it ~~might~~ have a high affinity (association constant) for the plasma protein relative to the displaced drug and/or must be in high enough free concentrations in the plasma (147). PTH, having a greater association constant and higher plasma concentration, satisfies the criteria and is expected to displace NPTH from the binding sites easily. The obtained displacement data illustrated neither the lower association constant drug (NPTH) nor the higher association constant drug (PTH) at various concentrations could have direct displacement effect. We therefore speculated that their binding sites on the plasma proteins might be different from one another or the binding of the two drugs involved was of non-competitive type.

6.5. CONCLUSION

Drug binding to plasma proteins may have an effect on its distribution, metabolism and elimination, which leads to pharmacodynamic consequences and therefore studies on the characteristics of drug binding to plasma proteins should be useful in interpreting pharmacokinetic data in clinical practice.

Equilibrium dialysis and incubation experiments were used to determine the binding of pethidine and its active metabolite, norpethidine, to isolated plasma proteins and human plasma. Drug-containing plasma samples or proteins solutions were dialysed against drug-free phosphate buffer (pH 7.4) in Teflon cells (DIANORM®, Zurich, Switzerland) separated by a dialysis membrane. Preliminary distribution tests showed that an equilibrium was attained within 4 hours at 37°C. The effects of drug concentration on protein binding was determined within the usual therapeutic range of pethidine (0.05-2.0 μg/ml) and norpethidine (0.025-1.0 μg/ml). The concentration of various protein solutions were prepared to encompass the expected ranges in human plasma. Plasma concentrations of pethidine and norpethidine in samples were assayed by a GC method which has been developed recently (Section 4.2.2., Chapter 4).

The results obtained showed that protein concentration-dependent binding exists in albumin and α_1-acid glycoprotein. The bound fraction of pethidine fell from 80 to 44% and from 77 to 31% with increasing pethidine concentration at 45 g/L albumin and 1.2 g/L α_1-acid glycoprotein

respectively. The bound fraction of NPTH fell from 69 to 18% and from 60 to 17% with increasing norpethidine concentration at 45 g/L albumin and 1.2 g/L α_1-acid glycoprotein respectively. The results of displacement experiment indicated that the binding of pethidine to plasma proteins was not affected by the presence of norpethidine and vice-versa. Therefore it could be concluded that there might have several non-competitive sites for pethidine and norpethidine or their binding sites might be different from one another.

CHAPTER 7

A COMPARATIVE STUDY OF PLASMA PETHIDINE AND NORPETHIDINE CONCENTRATIONS IN CAUCASIAN, CHINESE AND NEPALESE PATIENTS AFTER INTRAMUSCULAR POST-OPERATIVE PETHIDINE

7.1. INTRODUCTION

It is well recognized that the metabolism of some clinically used drugs not only varied among individuals of the same ethnic patient group, but also varied among different ethnic patient groups. For instance, the difference in the debrisoquine oxidation (148-151) is clearly evident in some population groups such that Chinese and Japanese have about 1% slow metabolizers whereas European Caucasians have about 9% poor metabolizers. On the other hand, the aromatic hydroxylation of mephenytoin (151) for Chinese and Caucasian is similar, but much higher among Japanese subjects. Other important classes which show interethnic differences include antihypertensive drugs, mydriatic drugs and antimalarial drugs. Several explorative studies on the metabolism of PTH under different urinary conditions (pH 2 to 4) among Caucasian, Chinese and Indian volunteers after a low dose of PTH have been reported (120,121,152,153). In those studies, Caucasian subjects appeared to hydrolyze and demethylate PTH more efficiently than Chinese subjects. Also, both Chinese and Indian subjects excreted significantly more NPTH in the urine while no difference was observed in the urinary recovery of unchanged PTH. It was concluded that PTH was mainly

metabolized by demethylation in the Oriental subjects, whereas Caucasian mainly eliminated the drug by hydrolysis under acidic urinary pH. Either routes of biotransformation are the major pathways of metabolism of PTH (Fig. 12, Chapter 4) and it is important to note that demethylation, an oxidative pathway, is dependent on cytochrome P-450 activity while hydrolysis is not. It is likely that the existence of an interethnic difference in the oxidative demethylation of PTH may be found in the PTH metabolism. These results prompted us to extend our study in Chapter 4 to include the investigation of a number of different ethnic groups to detect if there is an interethnic difference in PTH metabolism and disposition after a therapeutic dose. The difference in the metabolism of PTH was different between Oriental and Caucasian subjects (120-121) also provoked our interest in investigating the existence of interethnic variability in plasma binding of PTH and NPTH. Therefore, additionally, binding studies were carried out in plasma of the three patient groups from different origins who received a single intramuscular dose of PTH for post-operative analgesia.

7.2. EXPERIMENTAL

7.2.1. Chemicals and apparatus

Folin and Ciocalteu's phenol reagent was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Lowry reagent was freshly prepared by mixing anhydrous Na_2CO_3, 0.5M NaOH, 2% sodium amd potassium tartrates and 1% $CuSO_4$. for the determination of total protein concentration. Other chemicals and apparatus used in this experiment were the same as those listed in Section 6.2.1. in Chapter 6.

7.2.2. Patients study

After approval from the Research Ethics Committee of The Chinese University of Hong Kong, 30 patients who underwent various types of surgery (Table A23, p.205) were recruited from the British Military Hospital, Hong Kong. Informed consent was obtained from and close medical supervision were given to all patients before and during course of the experiment.

Caucasian (10 male; age, 25.6±4.4; weight, 74.1±6.5 kg), Chinese (10 male; age, 34.2±8.1; weight, 67.8±3.5 kg) and Nepalese (10 male; age, 26.3±1.9 yrs; weight, 66.1±8.0 kg), were given post-operative 1 mg/kg PTH intramuscularly (Table A23). Blood samples were obtained, via the ante-cubital vein with the aid of an indwelling 3-way stopper, at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12 and 24 hours after dose. All patients showed to have normal demographic data such as haemoglobin, plasma urea, creatinine, albumin and proteins. Plasma concentrations of PTH and NPTH were simultaneously

determined by a sensitive gas-liquid chromatographic technique as mentioned under Section 4.2.2., Chapter 4. A non-compartmental analysis, using statistical moment theory (Eq. 2 and 5, Chapter 1) were used to determined disposition parameters. Significance of differences of kinetic parameters among the 3 ethnic groups was evaluated using the Student's unpaired t test, with $p<0.05$ considered to be the limit of significance.

Plasma samples collected at various time intervals from each patient after the above pharmacokinetic studies were pooled together to give a bulked plasma samples. Since the bulked plasma samples from one Chinese and one Nepalese patients were not sufficient (less than 1 ml) for the binding study, only 28 (10 Caucasian, 9 Chinese and 9 Nepalese patients) out of 30 patients involved in the interethnic protein binding investigation. The patients' bulked plasma samples were stored at -20°C in a freezer before study.

7.2.3. Total Plasma Proteins Determination and Binding Study

The total plasma proteins in patients' samples were determined according to Lowry's assay (154). One millilitre of 1:400 diluted plasma samples (100 µl plasma in 3.9 ml distilled water) were pipetted into test tubes (10 x 80 mm) followed by the addition of 1 ml of freshly prepared Lowry reagent. The solutions were well mixed and allowed to stand for 15 minutes. Then 3 ml of 1:10 diluted Folin and Ciocalteu's phenol reagent in distilled water was added and the tubes were placed in a water bath set at 60°C for 15 minutes. The optical density (O.D.) was measured at 550 nm after the tubes

were cooled to room temperature. A reference blank and standards covered the calibration range of 10-100 g/L of total plasma proteins were prepared as above. The ~~O.D.~~ absorbance readings were converted to grams of proteins per litre by referring to the calibration curve. The procedure of the protein binding experiment was followed under the Section 6.2.3. in Chapter 6.

Details of calibration ?

7.3. RESULTS

7.3.1. Plasma concentration-time profiles of i.m. pethidine

The mean plasma concentration-time curves of PTH and NPTH after i.m. PTH dose (1 mg/kg) for the 3 ethnic groups were shown in Figures 26 and 27. Individual concentration-time points for the 10 patients in each of the groups were tabulated in Tables A24 to A29 (p.206-211). It appeared that the plasma concentrations of PTH and the time to reach its maximum plasma concentration during the early part of the experiment were almost the same among the 3 patient groups. However, Caucasians tend to eliminate PTH more rapidly than the other two groups. The N-dèmethylated metabolite appeared in the plasma varied from 0.25 to 1 hr after PTH administration. The mean time for reaching the maximum NPTH concentration was around 6 hrs in all cases with mean maximum plasma concentrations at 29.1, 25.4 and 26.1 ng/ml respectively for Caucasian, Chinese and Nepalese.

7.3.2. Comparison of pharmacokinetic parameters

Table 16 summarized the kinetic and disposition parameters of PTH and NPTH among the 3 ethnic groups. While no significant difference ($p>0.05$) was observed in the mean area under plasma concentration-time curves, Tmax and Cmax of PTH, the mean elimination half-life ($t_{1/2}$) of PTH and NPTH were higher in the Asian groups than the European. The average plasma clearance (Clp) of PTH was smaller in Chinese than Nepalese and Caucasian, although the difference between the two Asian groups was insignificant. The Clp of PTH was also significantly greater in Caucasian

(14.4±4.77 ml/minkg) than in the Chinese (9.96±2.93 ml/minkg) and Nepalese (12.6±2.91 ml/minkg) groups.

Fig. 26. The mean plasma concentration of pethidine in Caucasian (□), Chinese (△) and Nepalese (◇) after receiving a single intramuscular dose (1 mg/kg of total body weight) of pethidine.

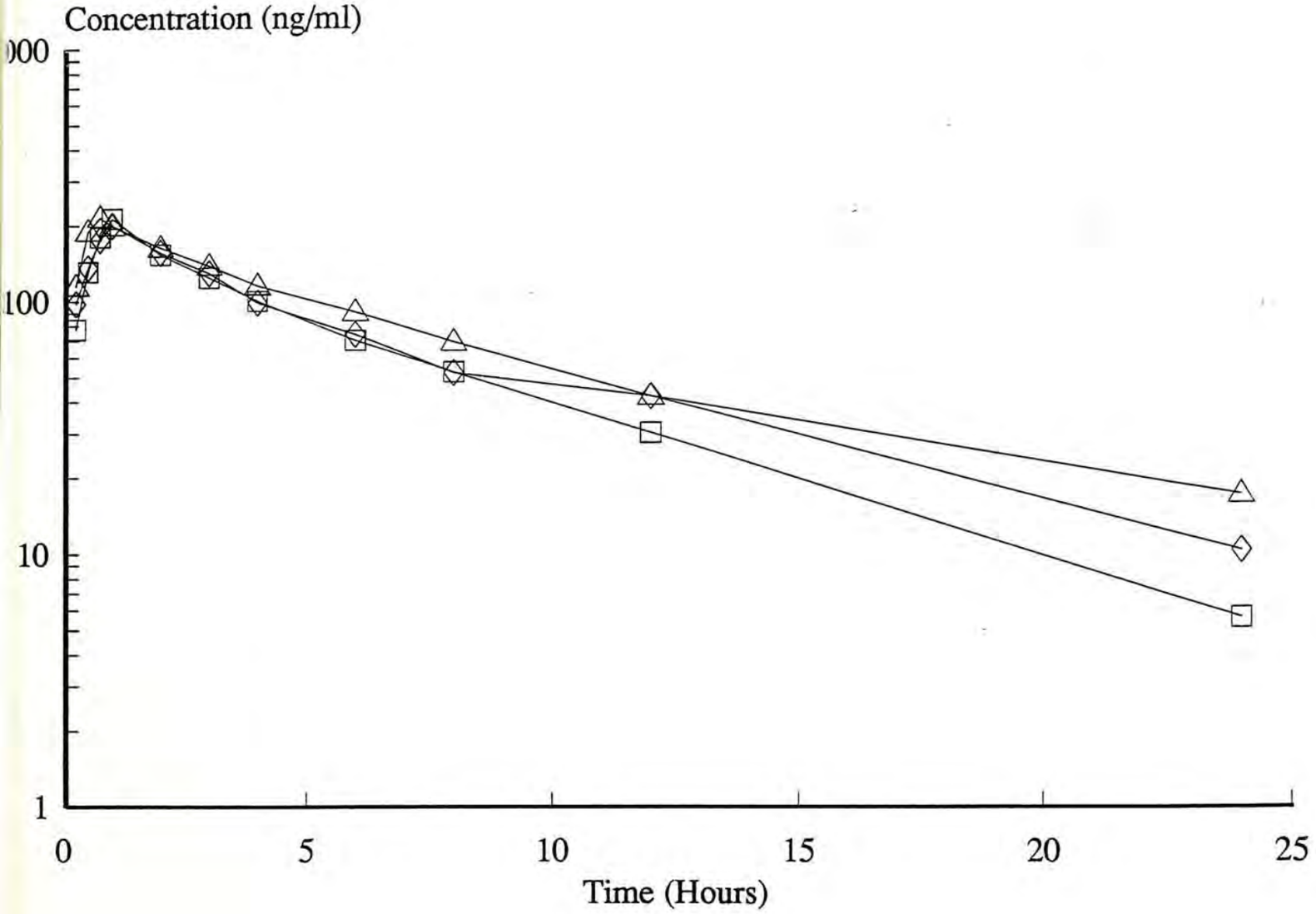


Fig. 27. The mean plasma concentration of norpethidine in Caucasian (□), Chinese (△) and Nepalese (◇) after receiving a single intramuscular dose (1 mg/kg of total body weight) of norpethidine.

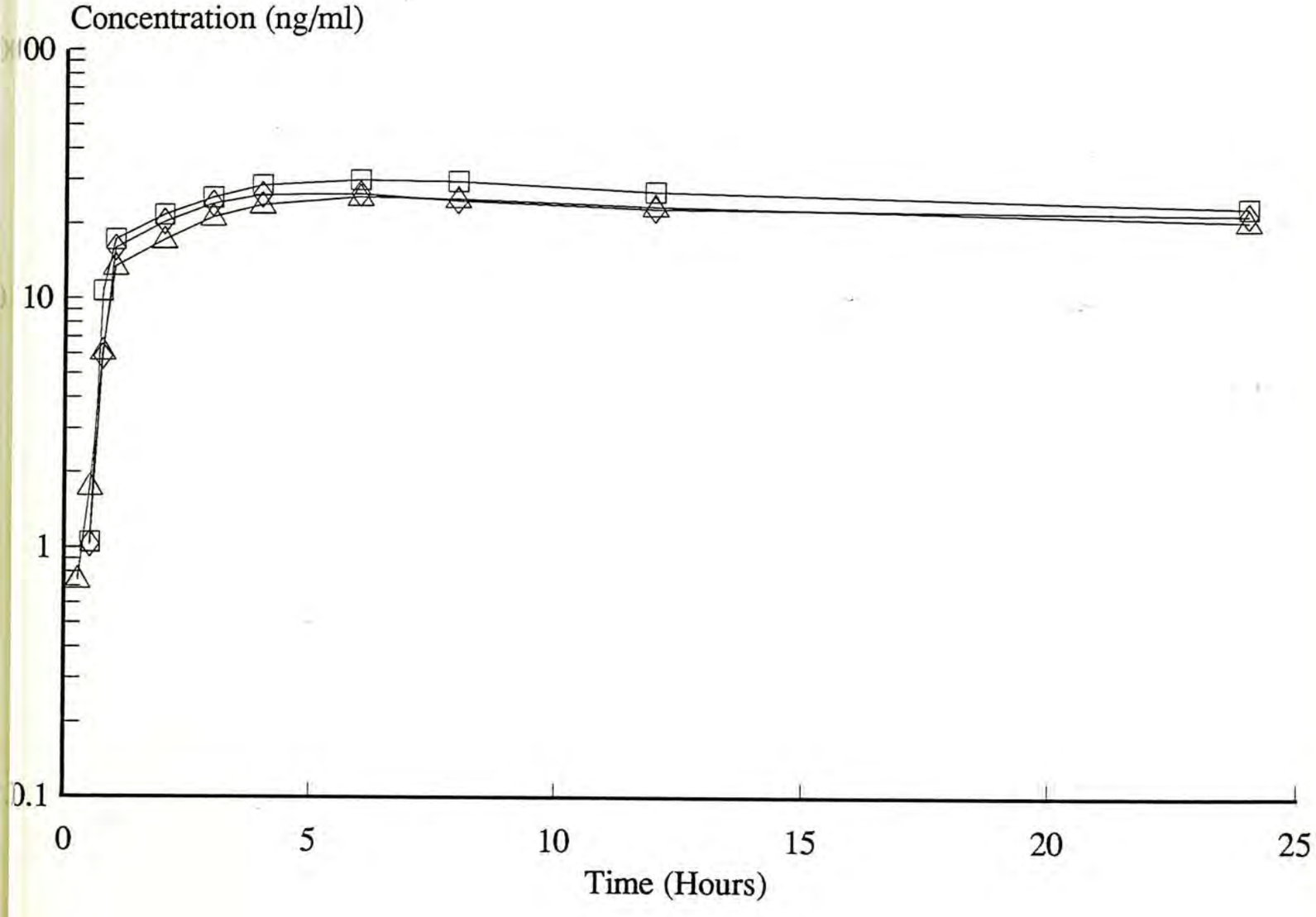


Table 16.

The pharmacokinetic data (Mean±S.D.) of PTH and NPTH in the 3 ethnic groups.

Patient Group	$AUC_{0-\infty}$ (nghr/ml)	Vss (L/kg)	Clp (ml/minkg)	Tmax (hr)	Cmax (ng/ml)	$t_{1/2}$ (hr)
Caucasian						
PTH	1287±415	5.08±1.16	14.2±4.77	0.86±0.13	228±66.2	4.53±1.32
NPTH	1864±751	----	----	5.50±1.72	31.0±4.85	39.1±15.3
Chinese						
PTH	1779±417	5.97±1.72	9.96±2.93	1.08±0.69	227±53.3	8.07±3.11
NPTH	2683±1880	----	----	5.70±1.64	26.0±5.53	71.0±50.2
Nepalese						
PTH	1399±364	6.04±1.76	12.6±2.91	1.14±0.50	218±79.1	6.15±1.22
NPTH	2676±1551	----	----	4.40±1.17	27.2±4.05	66.1±35.4

7.3.3. The interethnic binding study

Table 17 represented the total protein concentration in g/L and the percentage bound of PTH among the three patients groups. The protein concentrations of all patients were within the normal concentration rande (60-80 g/L). The values of protein binding were varied considerably and that the variation was mainly due to the different PTH plasma levels (ranging from 34.73 to 204.8 ng/ml) in the bulked samples. It was probably that patients with a higher PTH plasma concentration would have a smaller bound fraction than those with a lower concentration. Accordingly, the (mean±S.D.) % PTH bound at about 100 ng/ml for Caucasian, Chinese and Nepalese patients were 61.0±12.3%, 55.5±10.2% and 58.0±11.7%. However, the plasma levels of NPTH in all the bulked samples were low and beyond the detection limit of the assay (10 ng/ml), and therefore no information on this matter was obtained in the study.

Table 17.

Total plasma proteins and percentage of pethidine bound among the three ethnic groups.

Patient	Caucasian		Chinese		Nepalese	
	%PTH bound	protein conc (g/L)	%PTH bound	protein conc (g/L)	%PTH bound	protein conc (g/L)
1	58.43(102.6)	64.8	58.62(118.0)	78.0	67.23(63.54)	70.8
2	68.12(74.66)	70.0	66.72(58.89)	78.0	75.12(39.20)	70.0
3	59.24(88.88)	67.5	55.14(125.3)	69.0	61.52(87.33)	77.2
4	58.72(97.99)	71.7	39.23(188.7)	66.8	50.43(114.5)	73.8
5	35.43(197.5)	69.6	45.25(135.0)	77.6	42.38(143.8)	66.9
6	72.64(34.73)	71.6	70.12(55.52)	70.0	40.12(140.1)	79.0
7	65.00(66.27)	65.5	47.24(169.7)	73.1	63.92(125.4)	70.4
8	62.13(85.78)	74.7	54.24(76.12)	75.3	64.22(51.69)	73.9
9	80.12(28.71)	68.5	62.45(87.33)	69.0	56.93(83.77)	67.5
10	50.12(153.5)	70.0	------	------	------	------
Mean±S.D.	60.99±12.28		55.45±10.23		57.99±11.65	
	(93.06±50.93)		(112.7±47.24)		(94.40±38.50)	

Figures inside the parenthesis are the pethidine (ng/ml) plasma levels.

7.4. DISCUSSION

Diseases such as cirrhosis (155) and acute viral hepatitis (156) appeared to produce substantially similar decreases in plasma clearance and increases in elimination half-life of PTH. Patients involved in this study were carefully examined to ensure that none of them has developed these types of symptoms during the experiment. It was also reported that higher blood or plasma PTH concentrations in elderly patients (>70 yrs) compared with young volunteers (<30 yrs) and speculated a decreased clearance in the elderly subjects was responsible for those observations (157). In order to minimize this complication, the average age of the patients in the 3 groups were designed to be below 35 years old.

The rate of absorption of PTH in all patients was rapid and the peak plasma concentration was reached around 1 hr after dosing. No significant difference was observed in the mean time to achieve maximal absorption (Tmax) among the patients between Asian groups, but Caucasian (0.86 hr) seemed to absorb the drug faster than Chinese (1.08 hr) and Nepalese (1.13 hr). Similar results were reported (120-121) between Caucasian and Oriental groups (Chinese and Indian). The Vss obtained for Caucasian (5.08±1.16 L/kg) matched with the previous findings (110-111,155) but significantly lower than the other two groups, implying a greater distribution of PTH in the Asian groups. This can further be explained by the shorter $t_{1/2}$ in Caucasian group. As the drug was relatively less distributed, its presence in the plasma can be eliminated more quickly and readily. Therefore the Clp of PTH in Caucasian was greater but the AUC (14.2 ml/minkg; 1287 nghr/ml) was smaller than that of the Chinese (9.96 ml/minkg; 1779 nghr/ml) and Nepalese (12.6 ml/minkg; 1399 nghr/ml) respectively. These

observations were contrary to the published results (120-121) which stated Chinese subjects have higher Clp value than Indian but lower than Caucasian. This was possibly due to the different study design. In the previous reports (120-121), a 1/10 therapeutic PTH dose was orally administered by volunteers whereas in our study, a normal therapeutic i.m. dose (1 mg/kg) was given to patients.

The disposition data of NPTH among the groups were more clearcut. The AUC of Chinese and Nepalese was significantly greater than Caucasian, reflecting this N-demethylated metabolite tend to stay longer in the plasma of Asian groups or these two groups tend to metabolize PTH more readily via the N-demethylation route. These findings agreed with the hypothesis recently made (120). The difference of Tmax and Cmax among the 3 groups were insignificant. The elimination $t_{1/2}$ of NPTH varied substantially among individuals. Two Chinese and Nepalese patients gave extraordinarily high value (>90 hrs) and resulted in a mean $t_{1/2}$ (71.0 and 66.1 hrs respectively) greater than we obtained for a group of 5 Chinese patients in the previous experiment (39.6 hrs, Chapter 4). Despite these exceptionally large $t_{1/2}$, in general, the mean elimination half-life of the Asian groups were greater than Caucasian patients, suggesting the latter group eliminated NPTH more effectively than the former groups. The other important parameters such as Vss and Clp of NPTH could not be determined since this metabolite was generated via biotransformation after PTH dosing. The NPTH kinetic parameters have not been properly documented as yet and therefore, as mentioned earlier, it would be worthwhile to administer an acceptable dose of NPTH to healthy volunteers so that a more useful and accurate set of data could be obtained.

From this preliminary study, it appeared that both Chinese and Nepalese patients did not eliminate PTH as effectively as the Caucasian patients after a single i.m. dose and they produced more N-demethylated metabolite, NPTH, which stayed longer in the plasma of the Asian groups. It is likely that the interethnic difference in the PTH metabolism exists, although it can not be confirmed with certainty in this study. This statement can be further justified by performing more studies by increasing the number of patients in each ethnic group and recruiting patients from other races if possible. Since most of the major PTH metabolites are excreted in the urine, a study of their disposition by comparing the ratio of the unchanged drug to metabolites in the urine, could provide further information which may help to elucidate any difference in major metabolic route of pethidine among various ethnic groups.

In the investigation of interethnic binding difference, it was found that the amount of PTH in the bulked plasma samples, hence the percentage bound PTH, varied greatly (from 30.1 to 80.1%) among individuals within each ethnic group. Statistically, the mean % PTH bound among the three ethnic groups varied insignificantly ($p<<0.05$) which gave approximately 57% bound at 100 ng/ml. Therefore the presence of interethnic variability of PTH protein binding ~~was~~ possibly did not ~~to~~ occur, but we could not assume this type of phenomenon existing in other clinical drugs before any investigation is performed.

7.5. CONCLUSION

A comparison of the pethidine pharmacokinetics among the 3 different races (Caucasian, Chinese and Nepalese) showed that the time for maximum absorption, peak plasma concentration and elimination half-life of pethidine and norpethidine differed insignificantly after intramuscular dose of pethidine. However, the plasma pethidine clearance of Caucasian (14.3 ml/minkg) was greater than Chinese (9.96 ml/minkg) and Nepalese (12.6 ml/minkg). The total NPTH appeared in the plasma ($AUC_{0-\infty}$) of Asian groups was higher than that in the Caucasian group. It can be concluded that both Chinese and Nepalese patients did not eliminate PTH as effectively as the Caucasian patients after a single i.m. dose and they produced more N-demethylated metabolite, NPTH, which stayed longer in the plasma of the Asian groups. It is likely that the interethnic difference between European and Asian in the PTH metabolism is present and more work is required to potentiate and confirm the existence of the pethidine N-demethylation polymorphism.

The mean plasma levels of pethidine and the mean percentage of drug bound for Caucasian (n=10) were 93.06±50.93 ng/ml and 59.09±14.55% respectively, Chinese (n=9) were 112.7±47.24 ng/ml and 55.45±10.23% respectively and Nepalese (n=9) were 94.40±38.50 ng/ml and 57.99±11.65% respectively. This correlated well with the results from the experiment on the effect of pethidine on binding to plasma which gave 54.42±2.99% drug bound at a concentration of 100 ng/ml. Unfortunately, the concentration of norpethidine in the 28 investigated subjects were so low that we could not measure the percentage of pethidine binding.

CHAPTER 8

FUTURE PROSPECTS OF THE WORK

An analysis of the conclusions from the present investigation revealed that some further studies on the physicochemical properties of local anaesthetics and the disposition of pethidine in man would enhance the knowledge of the clinical pharmacology of these pain relief drugs.

Firstly, it would be of interest to investigate the effect of vasoconstrictors on the plasma concentrations of local anaesthetics other than bupivacaine, which are commonly used in the interpleural administration to produce post-operative analgesia. Since this type of drug monitoring is still limited, the results so obtained could provide useful information in handling these drugs more precisely and safely. As mentioned in Chapter 1, the pharmacokinetic parameters of a particular drug depend on its physicochemical properties. The work presented in Chapter 2 will therefore be more valuable if it is extended to include other local anaesthetics. Ideally, the number of drugs included in the investigation should be considerably large so that it can reveal the actual relationship of buccal absorption or lipophilicity for both amide- and ester-type local anaesthetics with respect to their pharmacologic activities such as the onset of action, duration of action and their potency. Such a study would require the development of a versatile analytical procedure, based on GC technique, for the identification and determination of a family of local anaesthetics in biological fluids.

Secondly, the results in the comparative study of plasma pethidine and norpethidine in Caucasian, Chinese and Nepalese showed an interethnic difference in the metabolism of pethidine after the patients receiving a single intramuscular dose of pethidine. It would be useful to continue the investigation by increasing the number of patients in each ethnic group and recruiting more patients from other races. This would further justify and possibly confirm the observations currently obtained. It is also a good alternative to follow the comparative study by comparing the pharmacokinetic profiles of pethidine and norpethidine and examining whether such interethnic difference in pethidine metabolism exist in intravenous administration or other administration routes apart from the intramuscular route.

Since most of the major pethidine metabolites are excreted in the urine, a study of their disposition by comparing the ratio of the unchanged drug to metabolites in the urine could provide useful information in the disposition fate of pethidine in man, which may help elucidate more clearly the difference in major metabolic pathway of pethidine among various ethnic groups. The analytical method presented in Chapter 5 will be a helpful tool in this type of research. But owing to its time-consuming sample treatment, an improved methodology may permit the determination of a large number of urine samples. It is practically useful to develop a direct HPLC method to measures these analytes in urine. As mentioned in Chapter 5, the simultaneous determination of all the major metabolites of pethidine is difficult because of their marked difference in chemical properties. The

barrier may be tackled if the acidic and basic metabolites are determined separately. The basic parent drug and norpethidine can be conveniently measured by the GC method described in Chapter 5. The acidic metabolites (PTA and NPTA) and neutral metabolites (PTC and NPTC) can possibly be determined by a normal phase HPLC, by injecting the urine sample directly into the system.

Other analgesics such as fentanyl, morphine and thiopentone (Chapter 4) are usually administered prior to the post-operative analgesia produced by pethidine. Finally, it is necessary to examine if these analgesic drugs have any displacement effect on the protein binding of pethidine and its metabolite, norpethidine, in the plasma, which in turn may change their pharmacokinetic properties accordingly.

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APPENDIX:

Table A1.

Patients data (mean ± S.D.).

	Plain	Adrenaline
Age (yr.)	49.0±14.8	46.3±11.2
Weight (kg)	58.2±11.7	58.3±9.4
Sex (M/F)	3/3	6/0
Anaesthetic time (min)	92.5±23.0	90.0±24.9

Table A2

Plasma levels (ng/ml) of bupivacaine in plain group patients.

	TIME (minutes)											
Patient	0	5	10	15	20	30	45	60	120	240	360	480
A	0	630	940	1200	1520	3040	2500	2220	1740	1140	770	610
B	0	1810	1890	2050	2330	2540	2330	1710	980	600	480	420
C	0	1000	1780	2000	2270	2050	1540	1260	1050	770	-----	480
D	0	540	880	980	1520	1440	880	820	600	340	-----	250
E	0	500	1150	2080	2460	3110	2620	1850	1440	930	-----	550
F	0	1660	3060	2930	2720	2560	1920	1250	640	380	-----	300
MEAN	0	1023	1617	1873	2137	2457	1965	1518	1075	693	625	433
S.D.	0	581	825	701	502	630	665	503	447	314	205	138
S.E.M.	0	251	337	286	205	257	271	205	183	128	145	56.3

----- no sample obtained

Table A3.

Plasma levels (ng/ml) of bupivacaine in adrenaline group patients.

	TIME (minutes)										
Patient	0	5	10	15	20	30	45	60	120	240	360	480
1	0	3220	2140	3460	3150	2460	1700	1660	1190	800	-----	580
2	0	1540	2280	2980	3240	3080	2860	2140	1260	780	-----	630
3	0	2660	2720	2840	2530	2410	-----	1300	960	520	390	-----
4	0	1640	2460	2960	2900	2750	2640	1890	910	500	-----	360
5	0	3290	3530	3270	3480	2650	1980	1320	620	400	-----	350
6	0	1820	3010	3320	3000	2840	2450	1960	1350	780	570	470
MEAN	0	2326	2857	3138	3050	2698	2326	1712	1049	628	480	478
S.D.	0	797	461	245	325	249	477	347	271	175	127	126
S.E.M.	0	325	189	99.9	133	102	213	142	111	71.4	90.0	56.5

----- no sample obtained

Table A4.

Pharmacokinetic data of bupivacaine in plain group patients.

Patient	Pharmacokinetic Parameter						
	AUC∞ (nghrml^{-1})	Vd (lkg^{-1})	Clp (mlmin^{-1}kg^{-1})	Tmax (hr)	Cmax (ngml^{-1})	t1/2 (hr)	Elimination Constant (hr^{-1})
A	13.6	0.995	183.7	30	3040	232	2.98x10^{-3}
B	9.80	2.14	255.0	30	2540	301	2.30x10^{-3}
C	10.6	1.80	236.2	20	2270	306	2.26x10^{-3}
D	5.17	2.70	791.8	20	1520	233	2.98x10^{-3}
E	12.2	1.22	204.7	30	3110	245	2.83x10^{-3}
F	7.90	3.47	316.5	15	2930	356	1.95x10^{-3}
MEAN	9.88	2.05	331.3	24.2	2570	279	2.55x10^{-3}
S.D.	3.03	0.928	230.2	6.65	606	50.4	4.37x10^{-4}
S.E.M.	1.24	0.379	93.98	2.71	247	20.6	1.78x10^{-4}

Table A5.

Pharmacokinetic data of bupivacaine in adrenaline group patients.

Patient	Pharmacokinetic Parameter						
	AUC_{∞} (nghrml^{-1})	Vd (lkg^{-1})	Clp (mlmin^{-1}kg^{-1})	Tmax (hr)	Cmax (ngml^{-1})	t1/2 (hr)	Elimination Constant (hr^{-1})
1	12.1	1.42	207.4	15	3460	276.8	2.50×10^{-3}
2	12.6	1.30	198.3	20	3240	264.1	2.62×10^{-3}
3	7.12	1.59	351.0	15	2840	169.5	4.09×10^{-3}
4	8.38	1.51	298.5	15	2960	195.9	3.54×10^{-3}
5	7.81	2.35	320.2	10	3530	258.9	2.68×10^{-3}
6	11.6	1.33	216.2	15	3320	243.7	2.84×10^{-3}
MEAN	9.92	1.58	257.4	15	3230	234.8	3.05×10^{-3}
S.D.	2.41	0.393	65.90	3.16	274	42.61	6.31×10^{-4}
S.E.M.	0.986	0.160	26.90	1.29	112	17.46	2.28×10^{-4}

Table A6.

The theorectical (in parenthesis) and buccal absorption of the six LA in two male volunteers.

	pH							
% absorption	3.0	4.0	5.0	6.0	7.0	7.4	8.0	9.0
Amethocaine	2.58	2.58	3.86	7.25	19.2	32.1	63.6	82.1
	4.35	4.51	8.86	15.0	20.1	34.5	55.9	74.2
	($4x10^{-4}$	$4x10^{-3}$	$4x10^{-2}$	0.35	3.43	8.18	26.2	78.0)
Bupivacaine	3.91	4.99	6.33	12.0	23.2	37.5	50.8	67.1
	2.83	3.37	4.85	9.05	21.3	36.0	53.4	69.8
	($9x10^{-4}$	$9x10^{-3}$	$9x10^{-2}$	0.88	8.18	18.3	47.1	89.9)
Etidocaine	6.18	5.78	7.16	19.1	34.7	46.1	60.0	73.9
	2.67	3.65	4.92	9.97	29.4	40.3	68.4	78.9
	($3x10^{-3}$	$3x10^{-2}$	0.50	2.45	20.1	38.7	71.5	96.2)
Lignocaine	7.63	6.21	8.76	16.2	21.9	24.4	37.4	62.1
	3.25	3.81	6.21	13.0	20.5	28.7	40.3	66.1
	($1x10^{-3}$	$1x10^{-2}$	0.11	1.11	10.1	22.0	52.9	91.8)
Mepivacaine	1.58	2.01	4.40	7.79	10.9	20.9	32.4	53.0
	4.76	3.70	6.10	8.49	11.5	22.5	35.6	56.5
	($2x10^{-3}$	$2x10^{-2}$	0.18	1.75	15.1	30.9	64.0	94.7)
Prilocaine	4.24	5.14	8.00	11.3	16.5	22.1	40.1	58.1
	5.14	7.97	10.4	11.4	18.1	24.7	38.2	57.8
	($1x10^{-3}$	$1x10^{-2}$	0.13	1.24	11.2	24.0	55.7	92.6)

Table A7.

The peak height ratio of drug in aqueous phase of control tube (at -20°C and 25°C) and various organic-buffer systems.

System	Peak height ratio of drug/I.S. in aqueous phase					
	Am	Bu	Et	Li	Me	Pr
Buffer (-20°C)	7.86	7.59	8.07	7.68	8.95	8.62
Buffer (25°C)	7.91	7.97	8.09	7.70	8.93	8.68
	7.90	8.00	8.10	7.52	9.05	8.15
	7.82	8.11	8.02	7.70	8.53	8.52
Hexane/buffer	1.40	0.475	0.150	2.55	5.90	4.20
	1.49	0.449	0.128	2.26	6.12	4.49
	1.50	0.529	0.147	2.67	6.04	4.97
Octanol/buffer	0	0.0204	0.0153	0.122	0.385	0.410
	0	0.0225	0.0168	0.168	0.385	0.413
	0	0.0239	0.0171	0.171	0.351	0.450
Ether/buffer	0.0233	0.0292	0.0205	0.192	0.539	0.354
	0.0210	0.0349	0.0233	0.105	0.526	0.262
	0.0207	0.0371	0.0251	0.112	0.561	0.287
Chloroform/ buffer	0	0	0	0	0.0461	0.105
	0	0	0	0	0.0393	0.123
	0	0	0	0	0.0427	0.122

Table A8.

The pH and volume of the expelled solutions after buccal absorption test.

pH of initial solution (vol.=25 ml)	Expelled solution; recorded pH and volume(ml)	
	Subject I	Subject II
3.0	3.46, 65	3.32, 50
4.0	4.26, 60	4.37, 55
5.0	5.54, 70	5.36, 45
6.0	6.20, 50	6.07, 40
7.0	7.03, 45	7.02, 40
7.4	7.41, 45	7.39, 40
8.0	7.78, 45	7.76, 40
9.0	8.84, 50	8.42,40

Table A9.

Plasma levels (ng/ml) of pethidine in 5 Chinese patients.

	TIME (hour)											
Patient	0	0.25	0.5	0.75	1	2	3	4	6	8	12	24
KLK	0	154	169	176	185	158	147	123	96.4	76.3	72.8	15.6
	(0	149	173	190	181	156	145	125	100	78.4	70.3	16. 2)
MMK	0	97.9	142	165	184	173	144	123	89.5	80.7	-----	18. 9
	(0	101	142	166	187	172	142	124	92.7	81.2	-----	16. 0)
KPW	0	95.9	273	303	249	128	70.2	64.5	55.3	57.2	25.8	17.7
	(0	97.6	280	304	246	128	72.6	62.5	56.7	56.8	26.1	15. 1)
MHL	0	158	251	326	193	153	144	120	65.2	64.3	46.1	30.7
	(0	166	236	320	184	151	152	121	60.3	70.3	47.4	32.7)
CHL	0	234	202	212	148	136	97.6	81.7	80.0	57.2	33.1	-----
	(0	241	208	206	145	136	99.8	77.9	78.1	53.9	36.2	-----)
MEAN	0	148	207	236	192	150	121	102	77.3	67.1	44.5	20.7
S.D.	0	56.5	54.7	73.8	36.4	17.9	34.9	27.5	17.0	10.9	20.7	6.79
S.E.M.	0	25.3	24.5	33.0	16.3	8.00	16.0	12.3	7.59	4.87	10.3	3.39

The data in parenthesis was determined by the reference method (103).

Table A10.

Plasma levels (ng/ml) of norpethidine in 5 Chinese patients.

	TIME (hour)											
Patient	0	0.25	0.5	0.75	1	2	3	4	6	8	12	24
KLK	0	0	0	11.6	14.8	17.0	21.1	21.5	25.1	26.2	25.0	20.9
	(0	0	0	9.73	15.6	16.7	24.1	22.6	24.8	28.4	22.2	21.8)
MMK	0	0	0	17.0	21.4	23.8	22.8	23.8	21.4	19.1	-----	12.7
	(0	0	0	14.7	23.7	23.1	25.1	24.2	21.7	19.8	-----	11.7)
KPW	0	0	10.6	14.3	13.3	18.5	25.0	32.2	35.3	34.5	21.0	14.1
	(0	0	8.69	17.1	13.8	18.0	27.2	30.6	35.9	35.4	24.2	15.6)
MHL	0	0	0	0	12.3	21.3	28.6	27.9	30.4	29.5	28.2	24.6
	(0	0	0	0	10.7	23.1	27.0	27.1	32.1	29.4	26.7	25.0)
CHL	0	0	0	0	15.1	20.0	26.9	27.2	28.3	27.7	26.1	-----
	(0	0	0	0	19.7	23.4	24.9	29.4	31.2	26.7	27.9	-----)
MEAN	0	0	2.12	8.58	15.4	20.1	24.9	26.5	28.1	27.4	25.1	18.1
S.D.	0	0	4.74	8.06	3.55	2.61	3.02	4.10	5.30	5.60	3.02	5.56
S.E.M.	0	0	2.12	3.61	1.59	1.17	1.35	1.83	2.36	2.50	1.51	2.78

The data in parenthesis was determined by the reference method (103).

Table A11.

Pharmacokinetic data of pethidine among 5 Chinese patients.

Patient	Pharmacokinetic Parameter						
	AUC_{∞} ($ng\,hr\,ml^{-1}$)	Vss ($l\,kg^{-1}$)	Clp ($ml\,min^{-1}\,kg^{-1}$)	Tmax (hr)	Cmax (ng/ml)	t1/2 (hr)	Elimination Constant (hr^{-1})
KLK	1996	4.92	8.35	1.00	185	6.90	0.100
MMK	1980	4.95	8.41	1.00	184	7.63	0.0908
KPW	1459	8.37	11.4	0.75	303	10.5	0.0660
MHL	2353	8.24	6.29	0.75	326	9.11	0.0761
CHL	1260	6.23	13.2	0.15	234	5.28	0.131
MEAN	1810	6.54	9.69	0.73	246	7.88	0.0928
S.D.	442.7	1.69	2.52	0.35	65.9	2.01	0.00251
S.E.M.	198.0	0.758	1.13	0.16	29.5	0.899	0.00112

Table A12.

Pharmacokinetic data of norpethidine among 5 Chinese patients.

Patient	Pharmacokinetic Parameter				
	AUC_{∞} (nghrml^{-1})	Tmax (hr)	Cmax (ngml^{-1})	half-life (hr)	Elimination Constant (hr^{-1})
KLK	2021	8.00	26.2	49.0	0.0141
MMK	854.5	4.00	23.8	24.0	0.0289
KPW	823.5	6.00	35.3	14.5	0.0477
MHL	2741	6.00	30.4	59.7	0.0116
CHL	2216	6.00	28.3	50.9	0.0136
MEAN	1731	6.00	28.8	39.6	0.0232
S.D.	856.0	1.41	4.38	19.3	0.0153
S.E.M.	382.8	0.632	1.96	8.64	0. 00686

Table A13.

The effect of pethidine concentrations on the plasma protein binding.

Conc. (μg/ml)	Percentage bound					
	1st	2nd	3rd	4th	5th	Mean±S.D.
0.05	72.65	69.43	75.21	74.24	68.97	72.10±2.81
0.10	52.43	50.62	58.14	56.25	54.67	54.42±2.99
0.25	49.42	46.73	53.66	50.21	45.13	49.03±3.30
0.50	42.77	46.24	39.86	44.72	45.18	43.75±2.51
1.0	39.24	43.77	46.73	42.85	43.62	43.24±2.68

Table A14.

The effect of norpethidine concentrations on the plasma protein binding.

Conc. (μg/ml)	Percentage bound					
	1st	2nd	3rd	4th	5th	Mean±S.D.
0.025	54.63	59.72	54.84	60.07	53.93	56.64±2.99
0.05	47.12	45.45	43.72	41.08	44.63	45.40±3.25
0.10	32.65	37.18	36.43	31.67	32.60	33.99±2.61
0.25	29.34	30.64	25.32	31.11	30.24	29.35±2.33
0.50	24.18	21.26	27.93	30.24	29.88	26.70±3.88

Table A15.

The effect of norpethidine concentrations on the plasma protein binding of pethidine*.

Conc. (μg/ml)	Percentage bound					
	1st	2nd	3rd	4th	5th	Mean±S.D.
0.01	45.07	39.73	38.25	42.87	41.44	41.47±2.66
0.02	36.02	35.98	40.73	39.11	40.68	38.51±2.38
0.05	44.89	43.90	42.00	37.76	39.52	41.61±2.97
0.10	33.98	44.87	37.64	41.45	43.88	40.36±4.53
0.20	47.39	37.27	39.07	46.42	45.29	43.08±4.59

* Pethidine concentration was kept at 0.50 μg/ml.

Table A16.

The effect of pethidine concentrations on the plasma protein binding of norpethidine*.

Conc. (μg/ml)	Percentage bound					
	1st	2nd	3rd	4th	5th	Mean±S.D.
0.05	37.09	31.25	33.67	33.01	36.72	34.35±2.50
0.10	29.79	30.96	35.25	34.11	32.05	32.43±2.24
0.20	27.68	34.23	30.55	31.19	33.57	31.44±2.61
0.50	36.14	30.97	31.22	34.75	34.62	33.54±2.31
1.0	28.76	32.09	36.32	34.77	33.46	33.08±2.88

* Norpethidine concentration was kept at 0.10 μg/ml.

Table A17.

The effect of pethidine concentrations on its protein binding to HSA (45g/L) and AAG (1g/L) (enclosed by parethesis).

Conc. (μg/ml)	Percentage bound					
	1st	2nd	3rd	4th	5th	Mean±S.D.
0.05	81.72 (72.86	79.83 80.91	84.77 77.14	77.25 75.66	78.09 81.27	80.33±3.02 77.57±3.57)
0.10	70.23 (57.84	67.48 65.66	72.14 62.19	65.92 64.78	66.81 59.27	68.52±2.59 61.95±3.39)
0.25	58.14 (49.18	53.72 42.66	52.63 50.91	55.11 43.26	56.72 48.08	55.26±2.22 46.82±3.67)
0.50	48.24 (35.72	53.14 40.18	45.67 37.63	52.62 33.24	49.18 35.01	49.77±3.12 36.36±2.65)
1.0	46.13 (30.14	42.19 29.83	39.82 34.77	43.77 31.86	43.96 32.64	43.17±2.34 31.85±2.01)
2.0	44.13 (29.73	48.53 30.14	42.46 33.63	47.12 32.77	40.23 30.88	44.49±3.38 31.43±1.70)

Table A18.

The effect of norpethidine concentrations on its protein binding to HSA (45g/L) and AAG (1g/L) (enclosed by parethesis).

Conc. (μg/ml)	Percentage bound					
	1st	2nd	3rd	4th	5th	Mean±S.D.
0.025	69.73 (62.53	70.28 67.43	61.72 58.15	73.79 55.63	67.41 56.72	68.59±4.47 60.01±4.87)
0.05	52.08 (42.73	55.74 48.64	48.26 39.56	45.19 45.12	50.68 46.37	50.39±3.97 44.48±3.48)
0.10	38.24 (31.97	31.73 28.14	33.69 25.66	35.00 27.08	30.26 25.04	33.78±3.08 27.58±2.74)
0.25	25.64 (24.32	27.93 21.08	29.14 19.75	30.98 20.00	26.15 24.87	27.97±2.19 22.00±2.43)
0.50	20.19 (18.09	21.28 15.73	23.77 17.22	18.65 20.64	19.72 19.05	20.72±1.95 18.15±1.85)
1.0	17.66 (16.72	18.72 12.64	14.98 18.33	20.02 17.28	18.92 17.95	18.06±1.91 16.58±2.29)

Table A19.

Pecentage bound pethidine (0.5 μg/ml) and norpethidine (0.1 μg/ml) (enclosed by parenthesis) as a function of HSA concentration.

Conc.(%)	Percentage bound 1st	2nd	3rd	4th	5th	Mean±S.D.
3.0	27.84	32.03	29.74	35.65	31.14	31.28±2.91
	(12.62	10.43	9.84	15.44	12.96	12.26±2.23)
4.0	42.93	37.64	45.66	35.73	40.24	40.44±3.98
	(20.44	18.13	22.66	17.43	21.11	19.95±2.16)
4.5	47.21	45.37	50.11	51.22	44.26	47.63±2.99
	(29.14	28.62	32.44	27.93	25.13	28.65±2.62)
5.0	48.67	53.42	51.13	52.65	47.98	50.77±2.39
	(36.11	28.64	32.13	26.17	30.94	30.80±3.74)
6.0	58.14	63.72	62.63	65.11	56.72	61.26±3.64
	(33.43	37.62	30.66	31.24	37.94	34.18±3.45)

Table A20.

Pecentage bound pethidine (0.5 μg/ml) and norpethidine (0.1 μg/ml) (enclosed by parenthesis) as a function of AAG concentration.

Conc.(g/L)	Percentage bound					
	1st	2nd	3rd	4th	5th	Mean±S.D.
0.5	19.26 (10.92	23.11 12.63	14.85 14.85	17.93 15.64	19.84 13.11	19.01±3.03 13.43±1.87)
0.8	28.34 (17.43	31.13 22.66	22.89 20.24	25.76 21.98	27.53 21.40	27.13±3.06 20.74±2.05)
1.0	35.13 (25.48	32.64 28,66	37.13 27.49	33.66 30.48	34.98 28.77	34.71±1.70 28.18±1.85)
1.2	40.13 (28.64	36.43 35.13	42.48 33.24	39.68 36.14	38.73 30.23	39.49±2.20 32.68±3.19)
1.5	52.19 (40.72	50.34 42.63	41.62 36.82	52.43 44.71	47.86 39.24	48.89±4.46 40.82±3.04)

Table A21.

Percentage bound pethidine to γ-globulin (12 g/L) and β-lipoproteins(5 g/L).

	Percentage bound					
Protein	1st	2nd	3rd	4th	5th	Mean±S.D.
γ-globulin	29.37	24.62	28.11	32.69	30.00	28.96±2.95
β–lipoprotein	29.73	27.67	22.43	23.15	20.67	22.73±3.08

Table A22.

Percentage bound norpethidine to γ-globulin (12 g/L) and β-lipoproteins(5 g/L).

	Percentage bound					
Protein	1st	2nd	3rd	4th	5th	Mean±S.D.
γ-globulin	20.64	21.73	16.97	19.23	21.76	20.07±2.02
β–lipoprotein	15.11	13.64	14.83	17.66	20.72	16.39±2.83

Table A23.

Clinical records of the patients in pethidine disposition study.

Name	Age	Race	Weight (kg)	Operation
Koon Kowk Leung (KLK)	35	C	68	Inguinal Herniorhaphy
Kwai Man Ming (MMK)	50	C	60.5	Inguinal Herniorhaphy
Wong Kim Ping (KPW)	36	C	68	Excision Exostosis
Lai Man Hin (MHL)	43	C	68	Excision Exostosis
Lam Ki Wing (KWL)	22	C	66	Urethrocystosis
Lau Chi On (COL)	34	C	74	Nasopharyngeal Biopsy
So Woon Hung (WHS)	30	C	69	Eua Sigmoidoscopy
Liu Hoi Fat (HFL)	25	C	65	Inguinal Herniorhaphy
Lau Kah Wah (KWL1)	32	C	70	Circumcision
Ip Kok Wing (KWI)	35	C	69	Excision Exostosis
Danks James (JD)	24	E	77	Frenuloplasty
Parks John (JP)	30	E	68	Vasectomy
Tanner Brian (BT)	21	E	80	Arthroscopy
Talman David (DT)	19	E	78	Circumcision
Davenport Anthony (DA)	28	E	77	Excision Exostosis
Moffat Alexander (AM)	27	E	62	Inguinal Herniorhaphy
Gable Stephen (SG)	23	E	79	Arthroscopy
Wood Charles (CW)	27	E	69	Biopsy Tibia
Gillett John (JG)	35	E	72	Cystoscopy
Weaving Mark (MW)	25	E	69	Arthroscopy
Gurung Kamalbdr (GK)	25	N	63	Lump Excision
Thapa Nirmalkumar (TN)	25	N	65.5	Gynaecomastia
Limbu Muniprasad (LM)	25	N	66	Dental Extraction
Rai Jangaraj (RJ)	25	N	59	Inguinal Herniorhaphy
Rai Gangaprasad (RG)	28	N	66	Arthroscopy
Gurung Prasad	25	N	64	Arthroscopy
Thapa Hareram (TH)	26	N	82	Arthroscopy
Gurung Ghanash (GG)	26	N	72	Dental Extraction
Magar Ranbahadur (MR)	31	N	52	Arthroscopy
Pun Kaubahadur (PK)	27	N	71	Dental Extraction

Table A24.

Plasma level (ng/ml) of pethidine in 10 Caucasian patients.

	TIME (hour)										
Patient	0	0.25	0.5	0.75	1	2	3	4	6	8	12	24
JD	0	101	104	218	227	130	64.8	51.8	46.4	22.0	17.5	0.00
JP	0	110	152	178	171	137	130	113	-----	-----	28.2	5.76
BT	0	83.9	148	210	175	162	102	83.2	67.9	50.5	36.1	7.04
DT	0	105	190	200	397	213	189	189	120	81.2	54.0	10.7
AD	0	104	185	187	250	201	189	116	56.4	37.5	-----	-----
AM	0	14.4	127	200	166	82.7	72.6	70.0	60.8	58.9	-----	5.37
SG	0	40.0	71.1	87.6	180	177	162	115	92.7	85.4	-----	9.22
CW	0	78.2	98.4	162	137	134	94.2	60.4	46.1	30.9	3.83	-----
JG	0	57.2	95.1	118	232	159	109	100	61.5	47.8	37.6	0
MW	0	82.1	137	240	197	134	122	104	75.5	55.3	32.4	6.38
MEAN	0	77.5	131	180	213	153	124	100	69.8	52.2	29.9	5.56
S.D.	0	31.4	39.2	46.7	73.3	38.1	44.4	38.9	23.9	21.2	15.9	3.86
S.E.M.	0	9.94	12.4	14.8	23.2	12.1	14.0	12.3	1.60	7.07	6.01	1.37

Table A25.

Plasma level (ng/ml) of pethidine in 10 Chinese patients.

	TIME (hour)										
Patient	0	0.25	0.5	0.75	1	2	3	4	6	8	12	24
KLK	0	154	169	176	185	158	147	123	96.4	76.3	72.8	15.6
MMK	0	97.9	142	165	184	173	144	123	89.5	80.7	-----	18.9
KPW	0	95.9	273	303	249	128	70.2	64.5	55.3	57.2	25.8	17.7
MHL	0	158	251	326	193	153	144	120	65.2	64.3	46.1	30.7
KWL	0	142	232	182	171	165	146	133	102	93.0	55.7	22.4
COL	0	112	214	216	240	203	169	125	95.1	71.2	45.5	17.6
WHS	0	119	208	237	199	142	110	97.3	77.0	64.4	31.7	10.5
HFL	0	56.6	107	135	165	116	87.9	76.3	55.9	38.9	16.3	9.44
KWL1	0	81.7	148	234	212	202	146	98.7	80.0	58.5	31.2	12.7
WKI	0	131	151	177	193	194	214	192	189	82.9	50.7	13.9
MEAN	0	115	190	215	199	163	138	115	90.5	68.7	41.7	16.9
S.D.	0	32.6	53.9	61.4	27.3	30.0	40.7	35.1	38.3	15.5	17.3	6.23
S.E.M.	0	10.9	18.0	20.5	9.14	9.99	13.6	11.7	12.8	5.17	5.75	2.08

Table A26.

Plasma level (ng/ml) of pethidine in 10 Nepalese patients.

	TIME (hour)										
Patient	0	0.25	0.5	0.75	1	2	3	4	6	8	12	24
GK	0	105	123	138	150	110	88.6	67.0	52.5	40.2	-----	10.9
TN	0	56.2	106	221	195	143	102	58.9	46.1	26.0	-----	12.6
LM	0	70.4	126	214	171	128	116	90.5	66.7	45.9	-----	9.15
RJ	0	103	133	145	149	160	137	113	64.0	16.6	-----	5.89
RG	0	66.5	81.7	110	152	146	131	91.5	79.7	62.6	-----	7.46
GP	0	74.1	97.3	128	152	116	90.3	83.6	62.8	44.6	23.8	6.50
TH	0	248	307	368	336	197	168	129	108	81.4	41.7	10.5
GG	0	42.6	98.6	104	139	207	188	137	115	91.2	59.2	20.5
MR	0	148	161	172	348	175	138	110	69.2	50.7	39.1	6.42
PK	0	61.0	125	163	211	175	132	103	71.9	59.6	44.9	12.5
MEAN	0	98.0	136	176	200	156	129	98.4	73.7	51.9	41.7	10.2
S.D.	0	60.6	64.0	78.1	78.0	33.0	32.2	25.2	22.3	22.9	12.7	4.37
S.E.M.	0	19.2	20.3	24.7	24.7	10.4	10.2	7.96	7.04	7.25	5.67	1.38

Table A27.

Plasma levels (ng/ml) of norpethidine in 10 Caucasian patients.

	TIME (hour)											
Patient	0	0.25	0.5	0.75	1	2	3	4	6	8	12	24
JD	0	0	10.5	17.3	19.3	22.6	24.7	28.1	27.2	26.4	23.9	16.0
JP	0	0	0	0	18.1	23.6	25.7	30.2	-----	-----	28.0	21.2
BT	0	0	0	14.2	19.2	22.6	25.3	28.7	32.0	30.5	27.0	20.9
DT	0	0	0	14.2	18.6	23.8	28.4	32.6	37.3	35.5	32.2	27.7
AD	0	0	0	11.9	16.5	19.3	23.1	-----	-----	28.5	-----	-----
AM	0	0	0	14.9	18.3	25.6	28.1	26.3	24.1	22.7	-----	17.7
SG	0	0	0	21.7	24.8	29.7	33.1	38.6	41.3	39.1	-----	28.0
CW	0	0	0	0	16.1	18.2	22.8	26.1	25.4	23.9	20.7	-----
JG	0	0	0	12.1	11.1	14.8	19.2	22.6	25.5	31.0	30.1	26.9
MW	0	0	0	0	9.77	16.2	22.8	23.7	26.8	25.8	23.9	20.7
MEAN	0	0	1.05	10.7	17.2	21.6	25.3	28.3	29.7	29.3	26.5	22.4
S.D.	0	0	3.31	7.87	4.27	4.53	3.86	4.69	5.97	5.40	3.98	4.62
S.E.M.	0	0	1.05	2.49	1.35	1.43	1.22	1.48	1.99	1.80	1.50	1.63

Table A28.

Plasma levels (ng/ml) of norpethidine in 10 Chinese patients.

	TIME (hour)											
Patient	0	0.25	0.5	0.75	1	2	3	4	6	8	12	24
KLK	0	0	0	11.6	14.8	17.0	21.1	21.5	25.1	26.2	25.0	20.9
MMK	0	0	0	17.0	21.4	23.8	22.8	23.8	21.4	19.1	-----	12.7
KPW	0	0	10.6	14.3	13.3	18.5	25.0	32.2	35.3	34.5	21.0	14.1
MHL	0	0	0	0	12.3	21.3	28.6	27.9	30.4	29.5	28.2	24.6
KWL	0	7.42	6.94	12.3	15.5	21.4	22.7	22.9	25.4	25.0	23.9	23.7
COL	0	0	0	5.95	9.72	12.7	18.4	24.7	27.7	27.0	26.1	24.7
WHS	0	0	0	0	10.9	13.5	14.3	14.0	13.1	12.7	12.7	10.9
HFL	0	0	0	0	8.64	7.22	13.7	17.7	20.7	21.7	25.3	23.7
KWL1	0	0	0	0	10.8	15.4	17.7	23.7	28.0	27.1	25.3	23.8
WKI	0	0	0	0	16.2	20.4	26.9	27.4	26.9	26.7	25.1	23.8
MEAN	0	0.742	1.75	6.12	13.4	17.1	21.1	23.6	25.4	24.9	23.1	19.8
S.D.	0	2.35	3.79	7.00	3.77	5.02	5.07	5.16	6.02	5.97	4.56	5.37
S.E.M.	0	0.742	1.20	2.22	1.19	1.59	1.60	1.63	1.91	1.89	1.52	1.70

Table A29.

Plasma levels (ng/ml) of norpethidine in 10 Nepalese patients.

	TIME (hour)											
Patient	0	0.25	0.5	0.75	1	2	3	4	6	8	12	24
GK	0	0	0	0	12.0	14.8	18.3	21.9	26.2	25.2	-----	23.7
TN	0	0	0	0	16.4	21.6	25.7	29.3	34.2	32.5	-----	29.7
LM	0	0	0	0	12.4	16.8	23.6	26.4	25.2	23.5	-----	18.0
RJ	0	0	0	10.4	16.2	18.3	21.7	25.4	24.5	23.1	-----	20.9
RG	0	0	0	0	14.7	16.8	19.3	20.8	19.6	18.8	-----	15.1
GP	0	0	0	16.4	19.2	23.1	27.2	19.7	28.6	26.8	25.7	24.0
TH	0	0	10.3	18.0	23.5	26.7	28.4	30.1	32.4	31.6	29.4	27.0
GG	0	0	0	0	13.6	19.2	23.5	25.9	23.9	22.4	21.0	19.9
MR	0	0	0	13.5	16.3	25.2	27.9	26.3	25.2	23.6	20.8	17.3
PK	0	0	0	0	14.8	19.3	23.1	22.8	20.6	17.8	16.0	14.1
MEAN	0	0	1.03	5.83	15.9	20.2	23.9	25.9	26.1	24.5	22.6	21.0
S.D.	0	0	3.26	7.78	3.40	3.89	3.50	3.25	4.63	4.79	5.12	5.09
S.E.M.	0	0	1.03	2.46	1.07	1.23	1.11	1.03	1.46	1.51	2.30	1.61

Table A30.

Pharmacokinetic data of pethidine among 10 Caucasian patients.

Patient	Pharmacokinetic Parameter						
	AUC_{∞} ($ngh\,rml^{-1}$)	Vss (lkg^{-1})	Clp ($ml\,min^{-1}kg^{-1}$)	Tmax (hr)	Cmax ($ngml^{-1}$)	t1/2 (hr)	Elimination Constant (hr^{-1})
JD	817.6	6.58	20.39	1.00	227	4.56	0.152
JP	1346	4.53	12.38	0.75	178	4.67	0.148
BT	1285	5.86	12.97	0.75	210	5.57	0.125
DT	2119	3.37	7.87	1.00	397	5.27	0.132
AD	1095	3.37	15.21	1.00	250	2.15	0.322
AM	1186	5.89	14.05	0.75	200	5.18	0.134
SG	1772	4.12	9.40	1.00	180	5.26	0.132
CW	696.8	5.48	23.92	0.75	162	2.05	0.338
JG	1244	6.25	13.40	1.00	232	5.60	0.124
MW	1309	5.31	12.73	0.75	240	4.97	0.140
MEAN	1287	5.08	14.23	0.86	228	4.53	0.175
S.D.	414.9	1.16	4.77	0.13	66.2	1.32	0.825
S.E.M.	131.2	0.368	1.51	0.042	20.9	0.418	0.261

Table A31.

Pharmacokinetic data of pethidine among 10 Chinese patients.

	Pharmacokinetic Parameter						
Patient	AUC∞Vd (nghrml^{-1})	Clp (lkg^{-1})	(mlmin^{-1}kg^{-1})	Tmax (hr)	Cmax (ngml^{-1})	t1/2 (hr)	Elimination Constant (hr^{-1})
KLK	1996	4.92	8.35	1.00	185	6.90	0.100
MMK	1980	4.95	8.41	1.00	184	7.63	0.0908
KPW	1459	8.37	11.42	0.75	303	10.5	0.0660
MHL	2353	8.24	7.08	0.75	326	16.0	0.0433
KWL	2052	5.21	8.12	0.75	182	7.63	0.0908
COL	1890	4.92	8.82	1.00	240	7.24	0.0957
WHS	1415	5.65	11.78	0.75	237	6.16	0.113
HFL	986.7	8.39	16.89	1.00	165	6.72	0.103
KWL1	1518	5.47	10.98	0.75	234	6.74	0.103
KWI	2141	3.59	7.79	3.00	214	5.18	0. 134
MEAN	1779	5.97	9.96	1.08	227	8.07	0.0940
S.D.	417.1	1.72	2.93	0.688	53.3	3.11	0.00248
S.E.M.	131.9	0.544	0.926	0.217	16.8	0.982	0.00783

Table A32.

Pharmacokinetic data of pethidine among 10 Nepalese patients.

Patient	Pharmacokinetic Parameter						
	AUC_{∞} (nghrml^{-1})	Vss (lkg^{-1})	Clp (mlmin^{-1}kg^{-1})	Tmax (hr)	Cmax (ngml^{-1})	t1/2 (hr)	Elimination Constant (hr^{-1})
GK	1160	8.13	14.37	1.00	148	7.86	0.0882
TN	1145	9.44	14.53	0.75	221	9.84	0.0704
LM	1276	5.74	13.06	0.75	214	5.66	0.122
RJ	1013	5.54	16.45	2.00	160	4.90	0.141
RG	1292	5.57	12.90	1.00	152	5.21	0.133
GP	1038	7.15	16.05	1.00	152	5.46	0.127
TH	1935	3.55	8.61	0.75	368	5.42	0.128
GG	2059	5.04	8.09	2.00	207	7.27	0.0953
MR	1501	4.33	11.11	1.00	348	4.81	0.144
PK	1569	5.87	10.62	1.00	211	7.10	0.0976
MEAN	1399	6.04	12.58	1.13	218	6.15	0.115
S.D.	363.6	1.76	2.91	0.0475	79.1	1.22	0.0249
S.E.M.	115.0	0.557	0.921	0.0150	25.0	0.386	0.00789

Table A33.

Pharmacokinetic data of norpethidine among 10 Caucasian patients.

Patient	Pharmacokinetic Parameter				
	AUC_{∞} (nghrml^{-1})	Tmax (hr)	Cmax (ngml^{-1})	half-life (hr)	Elimination Constant (hr^{-1})
JD	1098	4.00	28.1	24.0	0.0289
JP	1802	4.00	30.2	38.3	0.0181
BT	1484	6.00	32.0	29.3	0.0237
DT	2426	6.00	37.3	43.0	0.0161
AD	1720	8.00	28.5	37.7	0.0184
AM	1465	3.00	28.1	37.9	0.0183
SG	2123	6.00	41.3	32.6	0.0213
CW	954.5	4.00	26.1	23.0	0.0301
JG	3578	8.00	31.0	76.1	0. 00911
MW	1991	6.00	26.8	49.2	0. 00638
MEAN	1864	5.50	31.0	39.1	0.0190
S.D.	751.2	1.72	4.85	15.3	0. 00756
S.E.M.	237.6	0.543	1.53	4.84	0. 00239

Table A34.

Pharmacokinetic data of norpethidine among 10 Chinese patients.

Patient	Pharmacokinetic Parameter				
	AUC_{∞} (nghrml^{-1})	Tmax (hr)	Cmax (ngml^{-1})	half-life (hr)	Elimination Constant (hr^{-1})
KLK	2021	8.00	26.2	49.0	0.0141
MMK	854.5	4.00	23.8	24.0	0.0289
KPW	823.5	6.00	35.3	14.5	0.0477
MHL	2741	6.00	30.4	59.7	0.0116
KWL	7202	6.00	25.4	196	0.0354
COL	2506	6.00	27.7	60.2	0.0115
WHS	1248	3.00	14.3	61.0	0.0114
HFL	2256	8.00	21.7	68.8	0.0101
KWL1	3238	6.00	28.0	79.0	0. 00878
WKI	3932	4.00	27.4	98.2	0. 00706
MEAN	2683	5.70	26.0	71.0	0.0187
S.D.	1880	1.64	5.53	50.2	0.0138
S.E.M.	594.4	0.517	1.75	15.9	0. 00436

Table A35.

Pharmacokinetic data of norpethidine among 10 Nepalese patients.

Patient	Pharmacokinetic Parameter				
	AUC_{∞} (nghrml^{-1})	Tmax (hr)	Cmax (ngml^{-1})	half-life (hr)	Elimination Constant (hr^{-1})
GK	5489	6.00	26.2	145	0. 00479
TN	4883	6.00	34.2	97.8	0.00709
LM	1464	4.00	26.4	37.3	0.0186
RJ	2844	4.00	25.4	77.5	0.00894
RG	1406	4.00	20.8	45.8	0.0151
GP	2960	4.00	29.7	69.4	0.00999
TH	3346	6.00	32.4	69.0	0.0100
GG	2150	4.00	25.7	59.3	0.0117
MR	1257	3.00	27.9	31.1	0.0223
PK	958.3	3.00	23.1	29.1	0.0238
MEAN	2676	4.40	27.2	66.1	0.0132
S.D.	1551	1.17	4.05	35.4	0.00647
S.E.M.	490.6	0.371	1.28	11.2	0.00205

PUBLICATIONS

The following publications have been or will be published in advance of this thesis.

I. Abstracts:

1. O.W. Lau, K. Chan, Y.C. Wong, T. Gin, A. Kan and M. Gregory. Plasma levels of bupivacaine after interpleural administration. In the proceedings of the 11th Annual Conference of Hong Kong Neuroscience Society at the Chinese University of Hong Kong, August, 1989. *Neuroscience Letters*, **1989**; 37:S47.

2. Y.C. Wong, K. Chan and O.W. Lau. Chemical and pharmacological studies of some local anaesthetics. In the proceedings of the 11th Annual Conference of Hong Kong Neuroscience Society at the Chinese University of Hong Kong, August, 1989. *Neuroscience Letters*, **1989**; 37:S55.

3. Y.C. Wong, K. Chan, O.W. Lau, C. Aun, D.M. Lowe and I.T. Houghton. Plasma levels of pethidine and norpethidine in Chinese subjects after IM injection. In the proceedings of the 2nd Hong Kong Pharmacology Society Meeting in Association with the Jiangsu Pharmacolgical Society at the University of Hong Kong, December, 1989. *Asia Pacific Journal of Pharmacology*, **1990**; 5:P4.

4. K. Chan, C. Aun, I.T. Houghton, D.M. Lowe, O.W. Lau and Y.C. Wong. Plasma pethidine and norpethidine concentrations in Caucasian, Chinese and Nepalese patients after IM post-operative pethidine. In the proceedings of the 11th International Congress of Pharmacology at Amsterdam, The Netherlands, July, 1990. *European Journal of Pharmacology*, **1990**; 183:P1435-1436.

5. Y.C. Wong, O.W. Lau and K. Chan. Plasma protein binding of pethidine and norpethidine among three ethnic groups. In the proceedings of the 12th Annual Conference of Hong Kong Neuroscience Society at The University of Hong Kong, August, 1990. *Neuroscience Letters*, **1990**; 40: S20.

II. Original papers:

1. T. Gin, K. Chan, A.F. Kan, M.A. Gregory, Y.C. Wong and T.E. Oh. Effect of adrenaline on venous plasma concentrations of bupivacaine after interpleural adminstration. *British Journal of Anaesthesia*, **1990**; 64:662-666.

2. Y.C. Wong, O.W. Lau, K. Chan, C. Aun, D.M. Lowe and I.T. Houghton. Improved gas chromatographic method for the quantitation of plasma pethidine and norpethidine: application in determining pharmacokinetic parameters in Chinese patients after intramuscular administration of pethidine. *Journal of Biopharmaceutical Sciences*, **1990**; 1:267-276.

3. K. Chan, O.W. Lau and Y.C. Wong. Determination of pethidine and its major metabolites in human urine by GC. *Journal of Chromatography*, **1991;** 565:247-254.

4. Y.C. Wong, K. Chan and O.W. Lau. Protein binding characterization of pethidine and norpethidine and lack of interethnic variability. *Methods and Findings in Clinical and Experimental Pharmacology*, **1991**, in press.

5. O.W. Lau, Y.C. Wong and K. Chan. Simultaneous determination of six clinically used local anaesthetics in biological fluids. *Methods and Findings in Clinical and Experimental Pharmacology*, **1991**, in press.

6. O.W. Lau, K. Chan and Y.C. Wong. Gas-liquid chromatographic determination of bupivacaine in plasma: application in determining the pharmacokinetics of bupivacaine. *Forensic Science International*, **1991**, in press.